

# US ARMY MEDICAL DEPARTMENT JOURNAL



UNITED STATES ARMY  
VETERINARY CORPS

*July – September 2007*

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# Perspective

## Major General Russell J. Czerw

The US Army Veterinary Corps is the centerpiece of this month's issue of the *AMEDD Journal*. Although the majority of our Soldiers and Family members do not often come into direct contact with Army veterinary specialists, members of the veterinary service are part and parcel of all of our daily lives. Each day they can be found working in the field and laboratories in food science and food defense, performing research on communicable diseases and biological weapons, or ensuring the health of the military working dogs which have long been vital contributors to front line combat operations. Many readers will no doubt be surprised to learn of the extensive variety of skills and responsibilities, and the high levels of qualifications and education that are typical among our veterinary professionals. This issue of the *AMEDD Journal* provides a unique and long overdue look into the achievements these professionals have made over the years, and their continued invaluable contributions to the health and well-being of us all.

In this issue, the *AMEDD Journal* welcomes back BG Michael Cates, this time from his perspective as Chief, Army Veterinary Corps. BG Cates sets the stage for this issue as he clearly and succinctly describes the undeniable links between human health and the health of the animals that surround us. Indeed, at no time in history have the interrelationships of veterinary and human medical sciences been so well understood, and the extent of that knowledge is continually expanding. The fact that the Army Veterinary Corps is an integral component of the Army Medical Department, and has been for 91 years, is a confirmation of this relationship between the disciplines. BG Cates' article is an excellent overview of the roles Army veterinary specialists play, not only in support of US military missions, but on the larger stage of US foreign policy efforts to stabilize our turbulent world.

Understandably, the military is an organization that is in constant evolution as it adapts to changes dictated by society and technology, while at the same time contending with the dynamic nature of potential and active threats to the United States. Not surprisingly,



the Army Veterinary Corps' responsibilities are also changing in concert with those demands. In his concise, informative article, COL Gary Vroegindewey provides a clear description of not only the emerging roles, but also the expansion of current duties that Army veterinary personnel are performing in support of the Global War on Terror efforts of today.

The work of veterinary preventive medicine specialists is featured in 2 articles. In their contribution, MAJ Scott Hanna and his coauthors present an interesting history of the Army's food analysis laboratories and their operations today. They describe how those laboratories have changed from a Quartermaster Corps function for contract compliance and quality control, to their current position as keystones in the Veterinary Corps' force protection role of food safety and food defense. The increasing sophistication and formalization of testing practices and techniques, and the quick incorporation of emerging technologies are more examples of the Corps' adaptations to the ever-changing threats to our national security. Next, MAJ Jerrod Killian describes a research project focused on a serious, difficult-to-treat disease which is transmittable to both humans and dogs. Leishmaniasis is endemic in a large part of southern Europe and the middle east, areas where the US military deploys large numbers of military working dogs. MAJ Killian investigates the potential for the disease to be translocated to other

regions, especially the United States, where it is not currently a serious threat. This type of investigative work by our Army veterinarians is intensive, detailed, and absolutely vital in the management of diseases among our increasingly mobile global populations.

The use of laboratory animals in medical research is well understood as essential and invaluable. Less well known is the vitally important use of animals in the training of medical personnel. MAJ Craig Koeller's article provides insight to that element of training, which would not be possible without the Army veterinarian. He succinctly describes the protocols and procedures that must be followed during the planning and conduct of any training in which laboratory animals are used, and the veterinarian's responsibilities in ensuring strict compliance in every phase of that process. The veterinarian's support of this indispensable training contributes directly to human lives saved in the future.

Army veterinary pathologists are highly-trained professionals charged with the responsibilities of some of the most demanding and potentially hazardous areas of the Defense Department's biomedical research efforts. COL Keith Steele and his coauthors have contributed a comprehensive, important article describing this critical sector of Army veterinary medicine. Indeed, the tools and technology for biological warfare are very high on terrorist "want-lists" and represent a real and present danger to all mankind. The article shows how Veterinary Corps' experts have long been on the front lines of this very dangerous, albeit largely unrecognized theater of the Global War on Terror. Fortunately, Army veterinary pathologists are hard at work every day in this domain which is increasingly vital to our national defense.

Veterinarian Corps' professionals are often found in seemingly unlikely locations. MAJ Sam Yingst is one of those, and he describes his work as a comparative veterinary medicine officer at the US Naval Medical Research Unit No. 3 in Cairo, Egypt, as truly a "dream assignment." His work focuses on influenza, one of the most mobile and therefore potentially threatening forms of disease in today's highly mobile populations. Influenza diseases have been responsible for the most extensive and deadly pandemics in history. They are easily communicable among people, and as such represent a constant threat, either as naturally

occurring, constantly mutating, rapidly spreading diseases, or as bioengineered weapons. MAJ Yingst's article is a fascinating look at the diverse, dynamic, and rewarding work that not only requires keen scientific and analytic skills, but also mandates a global perspective and the ability to work with multiple, geographically and politically diverse agencies and individuals.

Veterinarians were first brought into military service to care for the numerous large animals required for armies to move and survive during campaigns: draft and cavalry horses, and cattle. As technological progress gradually eliminated the requirement for those animals, another animal became indispensable for US forces—the military working dog. Therefore, among the myriad other responsibilities of the Veterinary Corps, veterinary clinical medicine remains a primary function, and probably the one most familiar to the general military population. Two articles discuss different aspects of care of these invaluable dogs. CPT Kent Vince explores hip dysplasia, a chronic problem that plagues several breeds of large dogs preferred for military service. His detailed article describes the advances in detection, diagnosis, and treatments, especially the increasingly successful surgical repairs of the hip joint. The extension of the military dog's working life made possible by the surgery is of tremendous value, both in terms of the quick return of an experienced dog to service, and elimination of the expense of obtaining, training, and fielding a replacement animal. In the following article, COL Mack Fudge and his coauthors describe an effort to assist a US ally, the Republic of Korea, in dealing with a medical problem that afflicts their military working dog population. Eyeworm infestation is widespread within their dog population, and gradually reduces their military usefulness. The article details a classic clinical trial of the effectiveness of a drug which had been used for other animal species but had not been evaluated in a controlled study for dogs. This is another example of the initiative and dedication of Army veterinary specialists who continue to advance the art and science of their profession, both at home and abroad.

CW3 Gregg Burnham, a Veterinary Corps Food Safety Officer, and a team of highly skilled university researchers investigated the actual risk associated with raw meat which had been subjected to temperature

abuse in storage. This scientifically rigorous, complex study to accurately quantify that risk to consumers has the potential to significantly reduce costs of Defense Department meat procurement and distribution. Further, such research should inspire further investigations within the food safety community, both military and civilian. CW3 Burnham et al present their research in an detailed, clearly presented, and very informative article which demonstrates yet another way in which Army veterinary science is benefiting society as a whole.

In his article, CPT Christopher Young reminds us that the Army Veterinary Corps is well represented in the Reserve Component. The experience, qualifications, talent, and certifications these professionals bring into the Army are vitally important factors in sustaining the force readiness so important in today's extremely fluid mobilization environment. CPT Young's enlightening article is a testimony to the selfless dedication to service that is the hallmark of Army Reservists.

The true "jacks of all trades" in the Army Veterinary Corps are those veterinarians who serve in Special Operations Forces units. COL Robert Vogelsang provides us with a snapshot look at where they work

and what they do. His article describes the dynamic nature and often spartan conditions of their operational environment, and how their resourcefulness and flexibility are critical in mission success of their units.

LTC John Smith closes this issue of the AMEDD Journal with an excellent, "big-picture" discussion of the vitally important role Army veterinary specialists can and do play in support of US global efforts to improve stabilization of failed and failing economies. Instability in regional and national societies and institutions attract those interested in fostering calamity and sworn to the destruction of western civilization. Weak states are fertile breeding grounds for recruitment and training of insurgents and terrorist organizers. LTC Smith's article is a carefully researched, very understandable presentation of the situations and conditions which cause and sustain state instability, the analysis of the societal dynamics which must be addressed, and the role of the Army veterinarian in US efforts to reconstruct those economies and societies. This is an important look at both the present and the future of global security, a must read for all of us dedicated to the preservation of freedom and our way of life.

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#### **COL DEBBOUN JOINS THE AMEDD JOURNAL EDITORIAL REVIEW BOARD**

The *AMEDD Journal* welcomes COL Mustapha Debboun, MS, USA, as a member of the Editorial Review Board. COL Debboun is the Chief, Medical Zoology Branch, Academy of Health Sciences, AMEDD Center & School, Fort Sam Houston, Texas.

COL Debboun joins the board replacing COL George L. Adams, MS, USA, who has been a member of the board since April, 2006. We thank COL Adams for his dedication to the high standards and professional quality of this publication, and his years of service and support to the mission of the *Journal*.

*The Editors*



# **Healthy Animals, Healthy People: Inextricably Linked**

Brigadier General Michael B. Cates

Dogs and dolphins, monkeys and cats, horses and mules, rabbits, rodents, reptiles, and humans—multiple species, and all are part of the focused mission of the US Army Veterinary Corps. For over 91 years, officers in our Corps, along with support personnel, have been an integral part of the Army Medical Department, making critical global contributions toward the health of animals, as well as the health of Soldiers, Family members, and others. The US Army Veterinary Corps was formed in 1916 at a time when our country was just beginning to comprehend the relationship between animal and human health. We now know that those ties are tremendous. With extraordinary versatility and vigilance, our relatively small veterinary team of 3500 total personnel has continued its quest of the Army version of “One Medicine, One Health.”

## **ONE MEDICINE, ONE HEALTH**

Today, our nation’s medical and veterinary professions are working closely together in a resurgence of what we call One Medicine or One Health, that is, the recognition and appreciation for the linkage between human and animal health. The American Veterinary Medical Association and the American Medical Association have both taken formal steps toward better collaboration and partnerships, and veterinarians and physicians across the entire spectrum of their professions—in academia, private practice, government agencies, and many other aspects—have joined them.

The 19th Century German physician and pathologist Rudolph Virchow was one of the first medical professionals to connect animal and human health, stating:

Between animal and human medicine there is no dividing line, nor should there be. The object is different but the experience obtained constitutes the basis of all medicine.<sup>1(ppi)</sup>



BG Cates is the Chief, Army Veterinary Corps; the Commanding General, US Army Center for Health Promotion and Preventive Medicine; and the Functional Proponent, US Army Preventive Medicine.

Dr Calvin Schwabe, a veterinarian and epidemiologist, and a professor at medical and veterinary medical colleges, agreed, writing:

Impacts on human health are what most clearly delimit veterinary medicine’s world view and best define its broadly manifested importance as a profession.<sup>1(pp1-2)</sup>

This concept of One Medicine, One Health is embraced by the US Army Medical Department, and the Veterinary Corps is the cornerstone of its efforts.

## **HEALTHY ANIMALS**

When most people think of veterinary services, they think of the actual health care for animals. And, of course, the roots of military veterinary medicine were in animal care, beginning in 1776, when General Washington demanded a farrier for a regiment of horses. During the War Between the States, the requirement for adequate horse health continued, and the War Department provided each cavalry regiment with a veterinary surgeon. Later, congress required

that every applicant for these positions be graduates of a recognized veterinary college. After the Veterinary Corps' inception, with passage of the National Defense Act of 1916, equine medicine and surgery was a major aspect of our mission.

Through the many wars since World War I, the use of animals in the military has evolved, and with it, so has the health care of those animals. Today, most of what are usually considered "military working animals" are specialty trained dogs (eg, explosive detection, mine detection, narcotic detection, and patrol dogs), all helping our entire Department of Defense with force protection around the globe. Veterinary personnel provide medical and surgical care to those military working dogs wherever and whenever needed.

Our Corps also provides health care to horses, mules, marine mammals, service animals, and all animals involved with military biomedical research. These, when combined with pets of military personnel, total over 750,000, similar to the number of active duty Soldiers and Civilian employees of the entire US Army.

An additional, invaluable Veterinary Corps mission, more readily visible in recent years, is animal care for host nation countries—normally referred to as civil affairs or humanitarian assistance. During these deployments, veterinary support personnel provide clinical and preventive veterinary care to livestock and other animals of the native people in Afghanistan, Iraq, Nicaragua, African countries, and the Philippines, for example. We not only improve the health of the animals, but also directly impact the quality of life for the families and, many times, the economies of those countries.

The need for veterinary personnel in the US military began with animal health care requirements, and it remains relevant even in today's world. While the diversity of animals, as well as their use in our military, has changed over time, their health is an essential part of military medicine.

## **HEALTHY PEOPLE**

Shortfalls in Soldier health during the Spanish American War were pivotal in the evolutionary pathway leading to establishment of the Veterinary Corps before World War I. After thousands of alleged unnecessary casualties due to preventable illnesses, the country demanded that something be done to preclude such catastrophes in the future. The timing coincided

with wider acceptance of Virchow's views of animal and human links, and veterinarians were part of the solution.

Over 60% of disease pathogens and 75% of the emerging human pathogens are zoonotic, that is, transmissible between animals and humans. The Severe Acute Respiratory Syndrome (SARS) outbreak, the continuing Human Immunodeficiency Virus (HIV) epidemic and past influenza pandemics all originated in nonhuman species. Food and water-borne illnesses approximate a total of 76 million cases annually in the United States, and many can be traced to animal origins. This is especially true when the food commodities are animal by-products, such as milk, meat, and eggs. Protecting the food of deployed Soldiers, Sailors, Airmen, and Marines is a key mission for the Veterinary Service, whose members are deployed along with these forces. Bovine Spongiform Encephalopathy, or "mad cow disease," the spinach recall due to pathogenic *E. coli*, and the intentional melamine contamination of pet food are just a few examples which illustrate the necessity of having robust food safety and defense programs to mitigate the increased risks presented through consolidated food manufacturing systems and the globalization of food product distribution. Veterinarians are uniquely qualified to provide expertise in combating such outbreaks, not only because of our training in zoonoses, but also because of our "herd health" understanding and our systemic approaches to disease prevention and control.

To accomplish this, Army Veterinary Service personnel audit several thousand food producers in more than 80 countries annually, to ensure safe food for service members and beneficiaries. An example of the benefit to the combat commander was Veterinary Service approval of locally owned bottled water plants in Afghanistan at a savings of more than \$38 million per year and the elimination of over 4,000 water-delivery trips from supply routes, decreasing driver exposure to improvised explosive devices. These water plants are now part of the approved source audit program which is linked with other government food safety programs to share information, protecting service members and contributing to the nation's food safety.

One other significant example of zoonoses, with national and international prominence, is Avian Influenza. Army veterinarians have actively

## ***Healthy Animals, Healthy People: Inextricably Linked***

contributed to military and interagency planning processes. We have

- participated in developing the US Department of Agriculture (USDA) Avian Influenza Playbook in support of the National Response Plan,
- assigned Veterinary Corps Officers to the Joint Task Force-Civil Support,
- developed a capacity to respond,
- initiated training and equipment requirements to support the lead federal agency, and
- hosted interagency Avian Influenza surveillance and response conferences.

Veterinary personnel are currently an essential piece of overseas Avian Influenza testing and surveillance programs. In addition, we have trained over 150 veterinarians in the Department of Homeland Security Plum Island Foreign Animal Disease Diagnostician Course to support combat commanders in the field, as

well as the USDA at home. This capacity to respond to natural or agroterrorism emergency events was demonstrated by the deployment of veterinary personnel to support the USDA during the 2002 Avian Influenza outbreak in Virginia and Pennsylvania.

Veterinary Services also conducts programs to detect, prevent and control other zoonotic diseases, such as certain parasitic infections and rabies in pets of military personnel, as well as government-owned animals. Given the close association of owners with their pets and handlers with their military working animals, such programs are essential in the protection of the health of all.

The physiological and psychological benefits to humans from animals are not completely understood, but the human-animal bond is so strong that it sometimes transcends comprehension. Examples tying mental and physical well being of humans to their association with animals abound, in our country and



abroad, in civilian as well as military populations. Katrina victims refused to leave their homes without animals, and military noncombatant evacuations have stalled until pets could go too. Animal assisted therapy and visitation animals are important aspects of the Warriors in Transition program, in addition to inpatient programs in the Veteran's Administration and Department of Defense. Pets are considered important, inseparable parts of the family, and mascots are almost always desired among deployed units. Again, veterinary personnel positively impact both animal health and human health through their support.

Veterinary Service contributions to military medicine extend past food safety, animal medicine, and zoonotic disease control programs. Approximately 30% of Veterinary Corps Officers are specialty trained in laboratory animal medicine, veterinary pathology, or veterinary comparative medicine, and assigned to research and development positions. Their contributions in prevention span a wide spectrum of activities, from developing new-generation smallpox vaccines to malaria vaccines and prophylaxes, and evaluating Future Combat Systems for Soldier safety, from which the derived benefits extend from the Department of Defense to the nation to the world.

## INEXTRICABLE LINK

Veterinary Corps participation in all of our nation's conflicts since World War I has been an essential element in the maintenance of the health and well being of both animals and Soldiers. The highly technical education obtained by veterinarians has continued to prepare them for their changing mission requirements over the past 91 years, and we are uniquely qualified to contribute and lead in future efforts.

According to Zahn, Kaplan, and Steele, strategies related to One Medicine, One Health must span the entire spectrum of "veterinary and medical education, clinical care, public health and biomedical research."<sup>2</sup> Since 1980, the Army has been the Department of Defense Executive Agent for Veterinary Services,

providing veterinary support to all services, anytime, any place. Our veterinary missions, dictated in *Department of Defense Directive 6400.4*<sup>3</sup>—food safety and defense, animal medicine, zoonotic disease prevention and control, and medical research and training support—have been and continue to be inextricably linked to military human medicine.

Composed of 7 areas of concentration, the Veterinary Corps has over 750 veterinarians and warrant officers, and our entire veterinary team includes enlisted and civilian employees; active duty, reserve component, and Army National Guard; in Table of Organization and Equipment\* and Table of Distribution and Allowances<sup>†</sup> organizations. Recent events in the national and international food safety and zoonotic disease arenas underscore the criticality of continuing and even building enhanced veterinary capabilities.

I am very proud that this edition of the *AMEDD Journal* showcases examples of the breadth and depth of expertise, capabilities, and support missions of today's Army Veterinary Corps. It is an honor to be a part of this extraordinary team, as we continue to work diligently, with other members of the Army medical team, toward healthier animals and healthier people.

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\*Defines the structure and equipment for a military organization or unit.

<sup>†</sup>Prescribes the organizational structure, personnel and equipment authorizations, and requirements of a military unit to perform a specific mission for which there is no appropriate table of organization and equipment

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# *Emerging Roles of the US Army Veterinary Service*

COL Gary Vroegindewey, VC, USA

Since 1916 the US Army Veterinary Service has provided support to the warfighter through ensuring the safety of the military food supplies and providing care for government-owned animals. These are historic and enduring core functions that are critical to mission success of our fighting forces worldwide. In fact, the Army is the Executive Agent for the provision of veterinary medical care and services throughout the Department of Defense (DoD). The Surgeon General has delegated that responsibility directly to the Army Veterinary Service. In that role, the importance of medical research and development cannot be overstated. It is one of the 3 mission pillars supporting the overarching goal of Force Health Protection of all US military personnel and beneficiaries, and has been added as a primary role of the Veterinary Service.

In addition, recent national and global events have both refined the Veterinary Service's older missions and expanded its role into new areas. The new areas include disease surveillance; homeland defense; disaster response; food defense; and security, stabilization, transition, and reconstruction operations.

## **DISEASE SURVEILLANCE**

Historically, disease surveillance has been a passive system of reporting the incidence of specific diseases as they occur. Over 60% of all infectious diseases and 75% of emerging diseases are zoonotic, those diseases passed to humans from animals and animal vectors. Three events triggered the recognition for the need for active surveillance systems: Gulf War Syndrome, West Nile virus, and the avian influenza/pandemic flu.

Gulf War Syndrome in Soldiers and the inability to find a cause was the impetus for establishing a Gulf War Syndrome Study in Military Working Dogs. Since these animals are collocated in areas where environmental and disease exposure took place and have a shorter lifetime, they serve as a potential sentinel for human disease. The study was responsible, in part, for the establishment of the Electronic Medical Record for government-owned animals, and the creation of links among the deployment history,

medical findings, and necropsy reports to provide an active surveillance system. This DoD (Health Affairs) funded program has been approved and began development in 2007.

West Nile virus emerged as a potentially significant threat. This virus, which affects both people and animals, is an example of one of many emerging zoonotic diseases. As part of a national surveillance program, the US Army Veterinary Service provides active surveillance of horses owned by the military, and testing of ill and dying birds on installations nationwide.



An Army Veterinary Service food inspector examining military food supplies prior to loading.

The latest event is the current global avian influenza outbreak and potential pandemic flu threat. Army veterinarians are working on a range of planning, response, testing, and surveillance programs. To support the DoD requirements, a veterinarian with both a PhD in Public Health and an epidemiologic background has been assigned to The DoD Global Emerging Infections Surveillance and Response System (GEIS) to direct the surveillance of both human influenza and zoonotic diseases. Under the sponsorship of GEIS, the DoD Veterinary Service Activity hosted an International Avian Influenza Surveillance Conference in 2006, along with other training functions. Globally, Veterinary Service personnel assigned to the Navy Area Medical Research Unit-3 in Cairo, Egypt,\* are providing forward avian influenza surveillance in Africa and throughout the US Central Command's area of responsibility.

\*See related article on page 38.

## **HOMELAND DEFENSE**

Prior to the terrorist attacks of September 11, 2001, the Veterinary Corps had recognized the vulnerability of food to purposeful contamination. To address this issue, the Food and Water Safety Committee was established to evaluate

- current vulnerabilities,
- doctrine and policies to mitigate risk,
- emerging technology to detect and prevent contamination, and
- training required to meet these needs.

The Army Medical Department (AMEDD) Postgraduate Professional Short Course Program and the AMEDD Center and School Department of Veterinary Service developed a Food Safety and Security Course which is presented yearly for multiservice personnel, along with interagency representation from the Federal Bureau of Investigation (FBI), the US Department of Agriculture (USDA), the Food and Drug Administration (FDA), state public health personnel, commercial partners, and educational institutions. The course continues to be taught as part of the US Army Center for Health Promotion and Preventive Medicine's (CHPPM) annual Force Health Protection Conference. The committee also identified technology solutions for food defense that resulted in the acquisition and fielding of the portable food test sets as well as the Joint Biological Agent Identification and Detection System (JBAIDS) portable rapid polymerase chain reaction test sets.

Army veterinarians have served as liaisons to numerous activities in support of Homeland Defense. These include a public health veterinarian assigned to the Northern Command's Surgeons Staff; DoD Liaison to the USDA; support to the US Joint Forces Command's Joint Task Force-Civil Support; participation in White House working groups on agroterrorism; and work with the Government Coordinating Council, comprised of Federal, state, tribal, and local governmental agencies responsible for a variety of activities, including agricultural, food, veterinary, public health, laboratory, and law enforcement programs.

Strengthening of partnerships has been accomplished through programs for information sharing with FDA, Department of Homeland Security (DHS), and other federal agencies. These partnerships were proven valuable in execution of the recent pet food recall due

to melamine that also entered the human food supply chain and FDA canned chili product recalls.

Veterinary teams have deployed to support hurricane recovery efforts and events such as the 2002 Olympic Games in Salt Lake City, opening of the United Nations General Assembly, Republican and Democratic national conventions, the G-8 Summit, the presidential inaugurations, presidential funerals, and other events as part of our Defense Support to Civil Authorities.

## **DISASTER MANAGEMENT**

The role veterinary medicine plays in disaster management was highlighted in the aftermath of hurricanes Andrew, Floyd, and Katrina. After hurricane Andrew, the Veterinary Corps initiated an annual Veterinary Disasters course to prepare personnel to meet the requirements of national and international disasters. Working in conjunction with the Uniformed Services University of Health Sciences (USUHS) Center of Disaster and Humanitarian Assistance Medicine, and the Center of Excellence for Humanitarian Assistance and Disaster Relief, the course was developed to train personnel and encourage networking with other nongovernment agencies, government agencies, and academic institutions. Veterinary modules have also been developed and incorporated into the Combined Humanitarian Assistance Response Training (CHART) course, the Homeland Security for Healthcare Executives course, and in the USUHS humanitarian assistance curriculum.

## **FOOD DEFENSE**

Food safety, the detection and prevention of accidental food contamination, has been a core mission of the Veterinary Corps since its inception. Food defense, the prevention and detection of purposeful food contamination, has emerged as a Veterinary Corps responsibility which is shared with military security agencies. The Veterinary Corps has taken the lead and assisted in a wide range of food defense activities. In conjunction with CHPPM, the DoD Veterinary Service Activity wrote *Technical Guide 188*<sup>1</sup> and established food defense criteria which is used in the publication of the DoD Worldwide Directory of Sanitarily Approved Food Establishments for Armed Forces Procurement,\* DoD's audit program for commercial food establishments.

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<sup>1</sup><http://vets.amedd.army.mil/vetcom/directory.htm>

## ***Emerging Roles of the US Army Veterinary Service***

Veterinary Service personnel continue to participate in the DHS and FBI led Strategic Partnership Program-Agroterrorism to evaluate industry sector food defense vulnerabilities and develop industry standards to protect our nation's food supply. This is being done with the Veterinary Corps' veterinary officers and highly trained and experienced Food Safety Officers. The DoD Veterinary Service Activity, an Army Field Operating Agency, is working with FDA, USDA, DHS, and commercial partners to establish food defense guidelines and strengthen the safety of our national food supply. These food defense initiatives are further supported by Veterinary Service personnel assigned to support DoD agencies outside of the US Army Veterinary Command. These include key staff positions with the Defense Logistics Agency, Defense Supply Center Philadelphia, Defense Commissary Agency, Naval Supply Center, Army Center of Excellence-Subsistence, Army-Air Force Exchange Service, and others.

**Specialized Medical Assistance Response Teams – Veterinary** members have deployed as part of a multifunctional Food and Water Defense Team to support Army Chief of Staff high profile events for both food safety and defense.

The US Army Veterinary Service has participated in and led numerous exercises to train participants and test the capacity of veterinarians to support a wide range of national significant events, involving subjects from bioterrorism and agroterrorism to weapons of mass destruction.

## **SECURITY, STABILITY, TRANSITION, AND RECONSTRUCTION OPERATIONS**

*DoD Directive 3000.05*<sup>2</sup> identifies the security, stability, transition, and reconstruction (SSTR) activities as core DoD missions that "...shall be given priority comparable to combat operations...." The US Army Veterinary Service has been engaged in these

and similar activities over several years through providing Veterinary Readiness Training Exercises, Veterinary Civic Action Programs, and Cooperation Afloat Readiness and Training mission support through the Civil Affairs and Special Forces Veterinary Corps assets, plus TOE\* and TDA† personnel. While this is not a totally new mission, the emphasis and requirements for these activities have increased along with combatant commander recognition of the value of strengthening agricultural programs as a social, political, and economic stabilizing force. Combatant commanders' theater engagement plans increasingly focus on veterinary related programs.

This has resulted in an increase in both number and scope of deployments. The Reserve Component Veterinary Corps Officers<sup>‡</sup> provide the largest number of assets for these programs and are supported with active duty Veterinary Corps officers. Examples of these programs include rebuilding the Bosnian Veterinary College in Sarajevo, herd health programs in the Horn of Africa, avian influenza programs in Iraq, transboundary disease programs in Afghanistan, the establishment of national diagnostic laboratories, Medflag exercises in Africa, and other deployment activities.



An Army veterinarian examines a local farmer's donkey during a Veterinary Civic Action Program visit to a village in the Horn of Africa region.

In addition to the SSTR operations, the Veterinary Corps has been tasked to support Department of State Provincial Reconstruction Teams in Iraq, while continuing Civil Affairs support to brigade combat teams and the Multinational

\*Table of Organization and Equipment: Defines the structure and equipment of a military organization or unit.

†Table of Distribution and Allowances: Prescribes the organizational structure, personnel and equipment authorizations, and requirements of a military unit to perform a specific mission for which there is no appropriate table of organization and equipment

<sup>‡</sup>See related article on page 66.

Coalition-Iraq. To prepare for these expanding roles, the Veterinary Corps has initiated long-term health education and training opportunities including master's level training in humanitarian assistance, combined Master's of Public Health programs with the focus on international affairs and livestock management, as well as just-in-time training for deploying Veterinary Corps officers.

## SUMMARY

When leaving office, Tommy Thompson, the former Secretary of the Department of Health and Human Services, indicated that the 2 things that concerned him most were avian influenza and the safety of the United States food supply: "I, for the life of me, I do not know why the terrorists have not, you know, attacked our food supply, because it is so easy to do." These are DoD and national concerns and are a direct focus of the US Army Veterinary Service as part of its emerging roles and responsibilities.

While continuing its core missions of food safety, animal medicine, and research and development in support of the DoD, the US Army Veterinary Service

must be able to meet its responsibilities in the new emerging arenas. In order to meet these requirements, additional resources in the form of authorizations, training, equipment, and funding will be required. In addition, innovative partnerships and collaboration within the AMEDD, DoD, and interagency partners will be critical.

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# Army Veterinary Food Analysis Laboratories: Past, Present, and Future

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## ARMY VETERINARY LABORATORY SERVICE

The three pillars that form the core force health protection mission of the Army Veterinary Service are food safety, animal medicine, and research and development. Veterinary laboratory service has always played a key role in each of these areas, and continues to do so today. Veterinary officers hold vital positions in medical research laboratories, particularly in areas such as toxicology, virology, pathology, and laboratory animal medicine. Veterinary pathologists, clinical pathologists, and veterinary diagnosticians are essential elements in the health maintenance of military animals. Veterinary personnel in food analysis laboratories, the focus of this article, help provide the science that complements the art of food inspection.

Although an Army veterinary laboratory service has existed almost since the inception of the Veterinary Corps itself, its size has varied greatly throughout the years. In December 1917, the Army Surgeon General established a veterinary laboratory service to include 6 or 7 laboratory officers.<sup>1</sup> By the end of World War II, some 100 veterinary officers were serving worldwide in the veterinary laboratory sections of 32 Army medical laboratories.<sup>2</sup> Veterinary laboratory personnel have supported military conflicts throughout the latter half of the 20th century and beyond, from testing ice chlorination potability in Vietnam,<sup>3</sup> to providing food testing for Operation Iraqi Freedom. They also have a role in civil-military functions, whether supporting missions such as Joint Task Force Bravo in Latin America, or closer to home in the aftermath of Hurricane Katrina.

The number of veterinary laboratories has greatly decreased since 1945. Base Realignment and Closure, along with the advent of overnight shipping services, allowed various regional laboratories to be consolidated into the current Department of Defense

(DoD) Veterinary Food Analysis and Diagnostic Laboratory at Fort Sam Houston, Texas, and Veterinary Laboratory Europe in Landstuhl, Germany. Smaller food analysis laboratories in Hawaii and Korea cover those parts of the world that cannot quickly get samples to the 2 larger laboratories.

The mission of Army food analysis laboratories has also changed. Most of the early focus was on quality assurance testing and contract compliance for large stockpiles of subsistence. Dairy testing comprised a large portion of the food analysis laboratory work, as did can analysis and packaging testing of operational rations. The laboratories were often aligned under the Quartermaster Branch, until ultimately it was decided that food analysis is a medical mission, and the responsibility of the Veterinary Corps.

More recently, particularly with the advent of prime vendor contracts, food safety has become a main focus for the food analysis laboratories. Quality assurance checks are still performed, but more in the context of verifying the producer's own quality assurance program. Detection of harmful pathogens, toxins, and chemicals, and providing laboratory testing for foodborne illness outbreaks has taken priority. Also, the food analysis laboratories are becoming more and more involved with food defense, gaining expanded capabilities to quickly detect intentional contamination of subsistence with either traditional foodborne threats or with bioterrorism agents.

## CURRENT ARMY VETERINARY FOOD ANALYSIS LABORATORIES

The DoD Veterinary Food Analysis and Diagnostic Laboratory (FADL) at Fort Sam Houston is the Army's largest and most robust food analysis laboratory. The FADL is capable of performing a wide variety of microbiological and chemical tests on food

and water samples, supporting military and nonmilitary customers across the globe.

Food microbiology assays at the FADL run the gamut from basic, conventional microbiology using agar plates, to advanced, rapid techniques such as real-time polymerase chain reaction. The microbiology section tests for routine quality indicators as well as specific foodborne pathogens and toxins, screens operational rations for potential anthrax contamination, and tests samples associated with foodborne illness outbreaks. Although most of the samples submitted for microbiological testing come from North and South America, the section also confirms laboratory results for the surveillance laboratories in Hawaii and Korea when needed.

The food chemistry section's routine capabilities include the detection of pesticides in food and water and histamine in certain seafoods, heavy metal analysis, antibiotic residues in food and dairy products, and proximate analyses such as fat content of ground beef. FADL chemists are often called upon to identify foreign objects in food samples, and to respond to customer complaints. The chemistry section often receives samples from the other laboratories when more advanced chemistry testing is needed. New techniques currently under development include analysis for cyanide, better and faster ways to detect pesticides and heavy metals, and equipment and protocols to identify and quantify radioisotopes in food and water samples.

To ensure the validity of its results, the FADL is accredited through the American Association of Laboratory Accreditation (A2LA). This agency audits the FADL against the ISO 17025 standard, the General Requirements for the Competence of Testing and Calibration Laboratories. External A2LA accreditation provides further confidence in the accuracy and reliability of the FADL laboratory results, and allows the FADL to act as a confirmatory laboratory when necessary.

Due to the technical nature of many of the procedures at the FADL, most of the analysts are civilians. Military food inspectors (military occupational specialty [MOS] 68R) augment the civilians and perform many of the food microbiology and several of the food chemistry assays. This mix allows a consistent, stable workforce of highly trained civilians, while allowing the military food inspectors to receive

valuable training in food analysis techniques they can apply in future assignments.

A primary mission of the US Army's Veterinary Laboratory Europe (VLE) in Landstuhl, Germany, is to conduct microbiological and chemical analysis of food and bottled water for safety and wholesomeness. VLE is the only other accredited laboratory in the US military to conduct this testing, and employs conventional, rapid, and molecular methods to carry out these tasks in support of European Command and Central Command units. VLE customers include Army, Air Force, Navy, and State Department assets, with sample submissions from more than 40 countries.

Unlike the FADL, most of the food analysis technicians at VLE are junior enlisted personnel, MOS 68R. In addition to their Advanced Individual Training, they receive 6 months of training at the laboratory to learn the technical standing operating procedures and manuals, along with passing associated proficiency testing.

The Food Safety Laboratory at the Tripler Army Medical Center, Honolulu, Hawaii, performs microbiological screening for food samples from throughout the Pacific theater. They use a variety of rapid and miniaturized methods to perform this testing. The laboratory is staffed by a civilian microbiologist and a noncommissioned officer, MOS 68R.

Finally, the 106th Medical Detachment (Veterinary Services) Laboratory, Yongsan, in Seoul, South Korea, provides microbiological screening and limited chemical analysis of subsistence procured from the Korean peninsula. It is staffed by a civilian microbiologist, a Veterinary Corps officer, and MOS 68R personnel.

## **VETERINARY FOOD SURVEILLANCE LABORATORIES**

Despite the wide geographic dispersion of the fixed laboratories, many food products of interest are perishable, and the transport time may result in samples that are not testable. In addition, there are situations in which more immediate results are necessary due to operational considerations, and the time-sensitive nature may require a more expedient preliminary result. Difficulties shipping food samples across borders—from one country to another—may also exist, which delay or even prevent needed testing.

## Army Veterinary Food Analysis Laboratories: Past, Present, and Future

According to *Army Field Manual 4-02.18*:

Currently there is minimal testing that can be done at the deployed unit level. Suspect food samples are sent to the FADL in Fort Sam Houston, Texas, or to the US Army Veterinary Laboratory, Germany. In the near future the MDVS [medical detachment, veterinary service] will be able to screen food samples for the presence of foodborne [also water and ice borne] pathogens and biological warfare agents. If a pathogen or biological agent is detected through the screening process, food samples are collected and shipped to a confirmatory laboratory for further analysis.<sup>4</sup>

That day has finally arrived, with the fielding of 2 food testing sets specifically designed for use on the battlefield, in food production plants, storage facilities, and/or prime vendor facilities. Unit Assemblage (UA) 913A Veterinary Equipment Set Field Microbiology Diagnostic Kit will be used for rapid screening of food samples to assist in ensuring food safety. This kit features a handheld instrument which detects luminescence for adenosine triphosphate (ATP) associated with microorganisms and food/organic residues and pesticides. The Veterinary Service Support Team of the MDVS is authorized to use this kit. UA 914A Veterinary Equipment Set Food Testing Set will be used for rapid screening as well as presumptive results of food samples to assist in ensuring food safety and defense. This set features a small bench top analyzer that uses liquid scintillation counter for testing for aflatoxin and antibiotics and a bioluminescence counter for pesticides and ATP associated with microorganisms and food/organic residues. The Food Procurement Team of the MDVS is authorized to use this set.

Based on the needs, and knowing that fielding of the new sets was imminent, VLE initiated a training program, the Surveillance Food Laboratory Technician Workshops. The goal is to provide the training and references to allow a unit to stand up and run a local surveillance food and bottled water laboratory in support of the unit commander's mission.

While the purpose of the surveillance laboratories is to support the local command's mission, they are not yet designed to recover and identify pathogens. Instead, their role is to rule out problems based on indicator testing, or for referral to a reference laboratory such as VLE. Implementation of these laboratories has greatly enhanced local surveillance and destination

monitoring, and identified potential problems before those problems rise to a level that would affect the consumer.

Due to the number of training topics and the laboratory hands-on techniques to master, the workshops are designed with the low student-to-teacher ratio of 2:1 or 3:1. Training consists of a combination of lectures and laboratories, and topics include proper laboratory setup and maintenance, basic laboratory techniques, processing food and bottled water samples, reading and interpretation of results, followup actions to presumptive positive results, and reporting of results. The training emphasizes the use of rapid methods that can be used in garrison or field laboratories, including the following:

- Plating food products using Petrifilm™ plates (3M Corporation, St Paul, Minnesota), a ready-made culture medium system containing standard methods nutrients, a cold-water-soluble gelling agent, and additional chemicals or antibiotics as necessary for enumeration, selectivity, or differentiation of microorganisms.
- Testing of 100 mL subsamples of water, rinse, or diluted food samples using a single assay capable of selectively growing, detecting, and quantifying coliforms, *E. coli*, and hydrogen sulfide producing *Enterobacteriaceae* (such as *Proteus* and *Salmonella*).
- Use of a tabletop system for screening food and bottled water samples for pesticides and an indication of proper pasteurization. The system is also capable of screening for antimicrobials, aflatoxins, and other important chemical residues and indicators.
- Use of ATP swabs to determine the presence of ATP in water or on surfaces, reflecting the presence of organic material and level of sanitation in the sample. They are portable and rapid, and are carried on site into production plants, commissaries, and other areas where food is processed to determine the "cleanliness" of food processing surfaces. Results require only a 5-second luminescent reading in a portable counter.

The Veterinary Laboratory Europe has conducted 4 workshops so far, training just over 30 personnel. Army ranks have ranged from Private to Major, including Warrant Officers. Students have attended

from the European Command, Central Command, and Korea. In addition, classes have included Air Force Public Health Officers and a civilian involved in quality assurance at a meat processing facility.

In addition to the formal workshops, VLE has exported the training to the field during field training exercises to teach and demonstrate setup and operation of a food and bottled water laboratory in the field. In the last 3 years, VLE has provided subject matter experts and exportable laboratories for 3 different units on 3 field training exercises to meet requested needs.

The first formal training course has recently been conducted at the Department of Veterinary Science (DVS) at the Army Medical Department Center and School, a combined effort of DVS, the FADL, and VLE. The course was designed to support Veterinary Command personnel who are receiving one of the food analysis sets. Additional courses are planned, and the FADL is developing a proficiency testing program to ensure attendees maintain their skill set. DVS continues to update and develop training to ensure veterinary service personnel in TOE\* and TDA<sup>†</sup> units are trained to use the equipment.

Providing these workshops, taking the training to the field, and deploying subject matter experts are all critical components in ensuring the wholesomeness and safety of the food and bottled water supply for the US military. Access to onsite laboratory testing will allow commanders to make better informed, more timely decisions on whether to use a particular commodity, supplier, or producer.

## EMERGING TECHNOLOGIES

The food defense mission has also taken great leaps forward in recent years. With the increased potential for biological agent warfare and genetically altered



An instructor assists students in the examination of plate cultures as part of the Food Surveillance Laboratory Course at the Army Medical Department Center & School, Fort Sam Houston, Texas.

biological agents, the capability to confirm the presence of a biological agent has become essential to preventing casualties, thus maintaining effective combat power. One of the fastest ways to identify a biological agent is to determine if that agent's DNA is present. Polymerase chain reaction (PCR) has been used for years in fixed facility laboratories and is now available on the battlefield. In the mid-1990s, a joint service effort was started for the Joint Biological Agent Identification and Diagnostic System (JBAIDS) which uses PCR technology to identify and quantify biological warfare agents and other biological agents of operational significance for confirmatory and prognostic purposes. JBAIDS will perform specialized analytical tests on biological warfare agents or metabolites in environmental or food samples, samples or specimens from biological origins, or samples from military materiel.

Currently, JBAIDS only detects a select group of the 39 agents that can be potentially used as biological weapons. However, with the right sets of reagents, JBAIDS has the potential to detect any living organism

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that can cause illness in both humans and animals. Since JBAIDS is a joint service program, a centralized training program was also developed. Initial JBAIDS operator training for authorized military specialists consists of a 2-week course currently held at Brooks City Base, San Antonio, Texas. Before a unit can receive JBAIDS, 2 users from that unit must attend this training.

The first JBAIDS fielded to the Army went to the FADL. Army veterinary units that have or will receive JBAIDS are the Food Procurement Teams in the Medical Detachment, Veterinary Services, as well as select TDA units such as the FADL and VLE. Due to the highly complex nature of testing food samples, the FADL has been working on refining techniques and procedures taught in the JBAIDS operator's course, as well as developing methods to detect traditional foodborne pathogens using the JBAIDS platform. If such techniques can be validated, surveillance laboratories could begin looking for specific pathogens in food and water samples, in addition to quantifying indicator organisms.

Other technologies are currently being evaluated and developed by the FADL, the Natick Soldier Systems Center, other DoD agencies, the Food and Drug Administration, the Department of Homeland Security, and numerous other public and private organizations. The candidate technologies are diverse, such as electrochemiluminescence, which can detect foodborne toxins; immunomagnetic capture, which can separate a particular bacteria or virus from the other

organisms in the food; and microarrays, which can rapidly identify several potential pathogens using a single, small chip. As these become more mature, ruggedized, and exportable to the field, the surveillance laboratories will be able to provide a wealth of information about the microbial status of food and water samples in a very short amount of time.

## CONCLUSION

In many ways, Army food analysis laboratories have come full circle. While fast shipping once allowed a reduction in the numbers of laboratories, the desire for even faster results is now increasing their numbers once again. As technology has progressed, advanced testing protocols are now available to the surveillance laboratories, and more are sure to follow.

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# The Impact of Leishmaniasis on Military Working Dogs with Mediterranean Basin Exposure

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## ABSTRACT

### Background

Leishmaniasis is an infectious protozoan disease of people and domestic animals that occurs throughout temperate, subtropical, and tropical regions of the world. In the Mediterranean Basin, Canine Leishmaniasis (CanL) is endemic and might pose a risk to military working dogs (MWDs) stationed in the area. Concerns over translocating exposed MWDs into CanL nonendemic areas create the need to ascertain the impact of CanL in exposed MWDs.

### Objective

To determine the magnitude of CanL in exposed MWDs.

### Design

Serum/tissue examination of exposed MWDs using polymerase chain reaction (PCR) and immunofluorescence assay (IFAT) tests targeted to *L. infantum*; abstraction of MWD medical records for CanL-related signs.

### Setting

Military bases within the Mediterranean basin.

### Participants

Sixty-four MWDs located from a records search.

### Main Outcome Measures

PCR results; IFAT titers; frequency and number of CanL-related clinical signs abstracted from medical records; case definitions.

### Results

All PCR and IFAT tests were negative. No MWDs were classified as CanL cases or CanL probable cases. Although 16 MWDs met the CanL suspect case definition, no correlation was found between the length of time MWDs were exposed and the number of CanL-related clinical signs abstracted from medical records.

### Conclusions

The results suggest that the potential for MWDs to translocate CanL is very low.

## INTRODUCTION

Leishmaniasis is transmitted to humans and animals through the bite of the infected phlebotomine sand fly.<sup>1</sup> The disease is endemic in 88 countries, including all countries bordering the Mediterranean Sea with an estimated 12 million people worldwide affected,<sup>2</sup> and the number of cases has increased in the past decade.<sup>3</sup> *Leishmania infantum* is the etiological agent of human and canine visceral leishmaniasis (CanL) in the Mediterranean subregion. Domestic dogs are the main reservoir host in urban areas.<sup>4,5</sup> Leishmaniasis infections in both canine and human populations are most often nonfatal and asymptomatic, but can become fatal if left untreated.

Humans show a broad spectrum of responses to leishmaniasis. Some individuals display severe clinical

manifestations, yet others only show positive antibody titers. Clinical manifestations in humans are varied, but they include weight loss and intermittent spiking fevers. Leishmaniasis also presents a public health problem because it is difficult to treat. Existing chemotherapies are not wholly effective against the disease, and drug resistance is a growing problem.<sup>6</sup>

Canine leishmaniasis is endemic in the Mediterranean Basin. The seroprevalence of CanL ranges between 10% and 37%, and is even higher in isolated populations.<sup>7</sup> While leishmaniasis was once considered primarily a rural disease, it is increasingly found in suburban areas where small gardens create favorable conditions for the sand fly.<sup>6</sup> In Europe, where leishmaniasis is primarily a veterinary problem, estimates suggest that up to 7 million dogs are infected.<sup>8</sup>

## The Impact of Leishmaniasis on Military Working Dogs with Mediterranean Basin Exposure

In Mallorca, Spain, the prevalence of canine infection was determined by using polymerase chain reaction (PCR) and serological tests. The results indicate that 67% of dogs might be infected by leishmaniasis and that the prevalence of infection is much greater than the prevalence of overt leishmaniasis-related disease.<sup>7</sup>

Leishmaniasis can also display a wide disease spectrum in dogs ranging from clinical disease to asymptomatic infections.<sup>9</sup> However, successful treatment does not completely eliminate the CanL threat.<sup>10</sup> Even clinically cured dogs remain parasitologically positive and, therefore, infectious to the sand fly vector.<sup>11</sup>

Dogs infected with CanL that have failing immune systems can present a vague clinical picture with nonspecific and sporadic signs (see Figure 1). The most common clinical signs include ulcerated dermal lesions, dry skin with desquamation/scaling (lasting less than 2 months), lymphadenopathy, nephropathy, facial muscle atrophy, underweight at necropsy, and conjunctivitis. The incubation period of CanL can last from several weeks to several years, which makes immediate diagnosis difficult, especially if the dog is no longer in an area where leishmaniasis is endemic. These problems add to the long delay between sample collection, analysis, diagnosis, and subsequent control of outbreaks, making leishmaniasis difficult to eradicate.<sup>3</sup>



Figure 1. Rottweiler with leishmaniasis

The relationship between the prevalence of leishmaniasis in the canine population and human disease has direct public health implications.<sup>12,13</sup> The problem, in part, arises because clinical forms of CanL, characterized by chronic evolution of viscerocutaneous signs, occur in less than 50% of infected dogs.<sup>14</sup> Infected, asymptomatic dogs are sources of the parasite for the phlebotomine vector sand flies. Therefore, these dogs play an active role in the transmission of leishmaniasis,<sup>15</sup> posing a small risk of transmission from pets to members of the owner's family and other people in their community.<sup>16</sup> In addition, it has been suggested that infected dogs bitten by sand flies in nonendemic areas can spread the infection, creating new endemic areas.<sup>17</sup>

The risk for military working dogs (MWDs) to contract CanL while stationed in the Mediterranean Basin is undetermined. Typically, up to 60 MWDs are stationed at bases throughout the Mediterranean area. Some idea of exposure of MWDs to leishmaniasis can be gained from a seroprevalence study performed on 50 dogs, which resided with US personnel assigned to Naval Air Station Sigonella in Sicily. The data indicated a high exposure rate to CanL, with 60% of the study population having elevated immunoglobulin M antibody levels. These results suggest that the dogs were recently infected with *Leishmania infantum* during a 2- to 3-year tour in Sicily.<sup>18</sup>

The disease spectrum of leishmaniasis appears to correlate with the organism load level within individual dogs. Dogs with clinical disease have higher tissue and serum leishmaniasis organism levels. Testing instruments, in particular PCR and the immunofluorescent antibody test, more easily detect these higher organism levels. Conversely, dogs with a lower *Leishmania* burden have fewer organisms for diagnostic tests to detect and are consequently more difficult to diagnose.

In addition to the lack of widespread testing of MWDs, another major limitation is the inability to identify and count asymptomatic carriers, because classic diagnostic tests are insufficiently sensitive.<sup>2</sup> In addition, clinical signs are not reliable, making diagnosis of CanL difficult. These problems in diagnosing asymptotically infected leishmaniasis-positive dogs have prevented a clear assessment of the true risk of CanL to MWDs stationed near the Mediterranean Basin, although there have been no

known reported clinical cases of leishmaniasis in MWDs originating from that area. Accordingly, a reliable diagnostic test for the detection of CanL, both in symptomatic dogs and suspected animals, is needed.<sup>19</sup> The parasitological gold standard is isolation of the leishmaniasis organism, but parasites are rarely seen, and the histopathological method is nonspecific.<sup>20</sup>

Parasitological techniques currently in use, usually performed on bone marrow aspirates or lymph node aspirates, lack sensitivity to direct examination. Serological methods (immunofluorescence assay, enzyme linked immunosorbent assay [ELISA]) are effective in detecting active leishmaniasis when large amounts of specific antibodies are present. However, the usefulness of these techniques is limited in the areas where the disease is endemic due to the high number of animals with low levels of antibodies.<sup>21</sup>

The purpose of this study was to determine the prevalence of CanL in MWDs stationed at selected locations in the Mediterranean Basin using immunofluorescence assay testing (IFAT), PCR tests, as well as medical record abstraction and analysis. Public health concerns regarding leishmaniasis infections were also addressed.

## METHODS

### Study Population

Records, sera, and tissue samples were drawn from MWDs stationed in the following leishmaniasis-endemic locations:

- Iraklion and Souda Bay Naval Station, Crete
- Nea Makie, Greece

Egypt

Italy

- Sigonella Naval Air Station, Sicily
- Naples Naval Air Station
- Vicenza Army Base
- Palmenola Air Base

Spain

- Zaragoza Air Base
- Torrejon Air Base
- Rota Naval Air Station

Turkey

- Incirlik Air Force Base
- Ankara

### Sampling and Testing Protocol

A search was conducted to identify MWDs stationed at the selected military installations using the MWD database at the Department of Defense Dog Center, Lackland Air Force Base, San Antonio, TX. Of the 2,315 MWD medical records available, 64 matched the criteria. However, the medical records of 7 MWDs could not be located, resulting in 57 records for data abstraction. Frozen serum was available for only 32 (56%) of these 57 MWDs. Because post-Mediterranean basin exposure serology samples were used, 25 MWDs were identified as either missing serology records after CanL exposure, or missing frozen serum.

Thirty-six medical records had corresponding tissue samples available at the Walter Reed Army Institute of Research (63%). Tissue samples for 21 MWDs were unavailable, because no necropsy was performed or the medical record did not contain a pathology report from the Armed Forces Institute of Pathology (AFIP) in Washington, DC. Figure 2 illustrates how the study population was identified, and the procedures for obtaining tissue (PCR), serum (IFAT), and medical records.

Serological testing was performed by Frank Seuter, utilizing methodology developed at the Centers for Disease Control and Prevention (CDC) by Dr Peter Schantz, Division of Parasitic Diseases. Immunofluorescence assay testing was configured to detect specific antibodies to the leishmaniasis organism. The intensity of the titer reflects both the stage of the infection and the animal's response to the infection, with the antibody titer generally increasing (from 1:16 to 1:512 or greater) as the infection progresses.<sup>22</sup> The interpretation of the IFAT closely followed the CDC's recommendations: leishmaniasis-positive serum titers of 1:16 or higher were termed possible infections, titers of 1:64 or greater were termed highly suspected infections.

Fluorogenic polymerase chain reaction (Smart Cycler PCR cycling protocol) tests were performed with enhanced specificity, as the assay target incorporated a segment of the small-subunit rRNA gene, which is conserved among all leishmaniasis species.

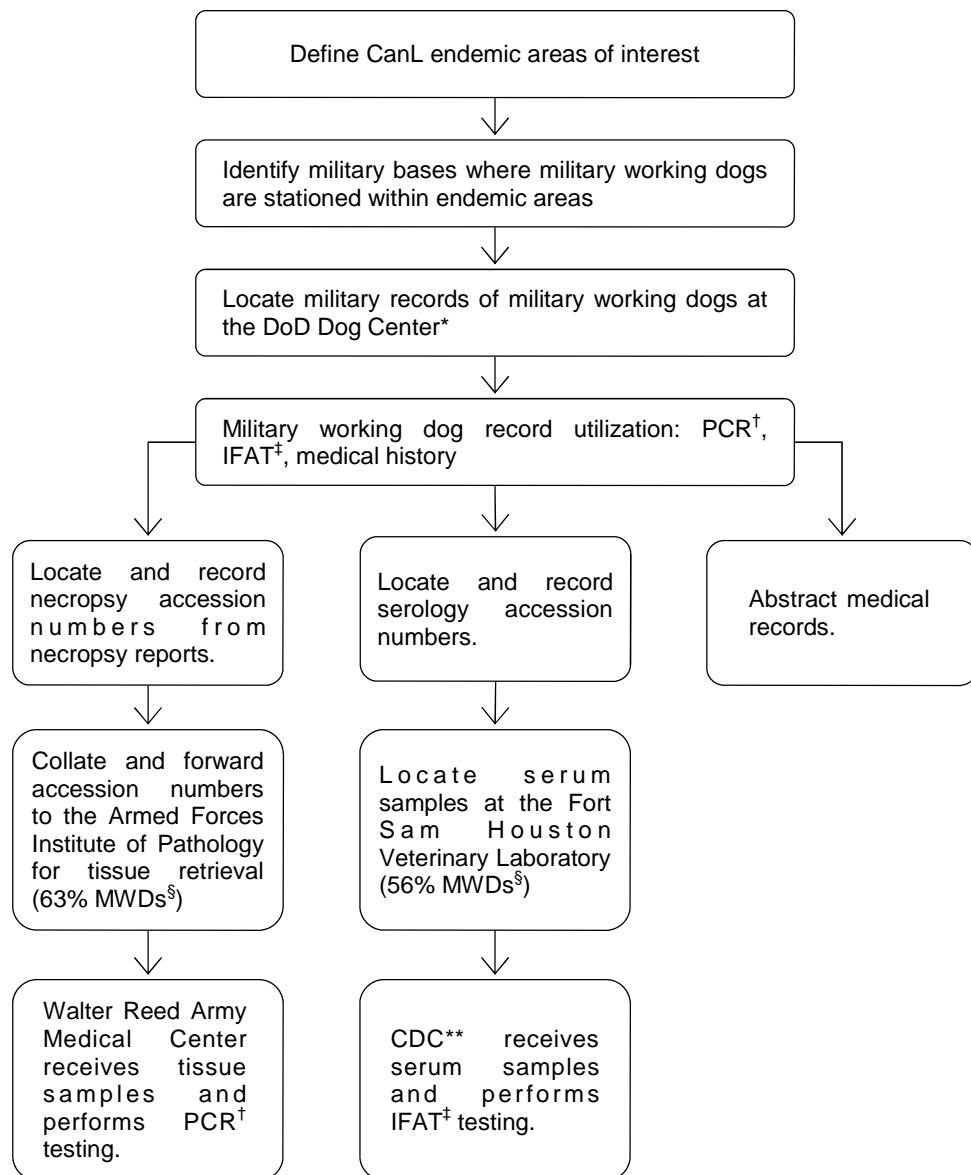


Figure 2. Process flow of military working dog tissue sampling strategy

\*Department of Defense Dog Center at Lackland Air Force Base, Texas

<sup>†</sup>Polymerase chain reaction

<sup>‡</sup>Immunofluorescence assay test

<sup>§</sup>Military working dogs

\*\*Centers for Disease Control and Prevention, Atlanta, Georgia

Sections (5  $\mu$ m thick) were cut from the samples, while paraffin removal, DNA purification, and DNA extraction (by column chromatography) were performed according to the manufacturer's instructions (QIAamp Tissue Kit; Qiagen, Valencia, CA). Specific PCR reaction mixtures incorporated water, MgCl<sub>2</sub>, PCR beads (formerly Amersham Pharmacia Biotech, Inc., Uppsala, Sweden, now Amersham Biosciences), primers, and the leishmaniasis probe. A *Leishmania infantum* probe was used for PCR testing, because it was the dominant leishmaniasis subspecies found in the Mediterranean Basin. Samples for PCR testing were run in groups of 10-13.

## MWD MEDICAL RECORDS

Medical records were available in either hardcopy or microfiche format. The study focused on the MWD medical record's Master Problem List (Form 3071), standard form 600 (SF 600), and the record of military dog physical examination (DD Form 1829). The records summarized significant diagnoses, and aided in the collation of CanL-related medical problems. Laboratory and necropsy reports within the medical records enhanced the clinical picture.

## DATA ANALYSIS

Questions concerning the medical history of MWDs were designed to determine the presence or absence of specific CanL-related clinical signs. The characteristics of MWDs in each category were collated to derive the corresponding percentages for MWDs having designated signs and symptoms. This classification strategy allowed the identification of potential CanL-positive and negative MWDs using the relative frequencies of observed signs and symptoms. An abstracted list of variables, including specific clinical signs, is presented in the Table.

Information was recorded in an Excel (Microsoft Inc, Redmond, WA) spreadsheet and stored for data analysis. Minitab Statistical Software (Minitab Inc, State College, PA) was used for data and statistical analysis. The correlation of exposure months and number of clinical signs was tested using the Pearson correlation coefficient.

Abstracted list of variables collected from military working dog medical records to use in data analysis to determine the presence or absence of specific CanL-related clinical signs.

Demographic Variables	Medical History Variables
Name	Ulcerated dermal lesion(s)
Tattoo number	Skin desquamation/scaling
Date of birth	Underweight on necropsy
Date of death	Facial muscle atrophy
Age at death	Nephropathy
Breed	Lymphadenopathy
Gender	Conjunctivitis
Number of times reporting to Mediterranean Basin	Leishmaniasis screening
Months in Mediterranean Basin	Leishmaniasis diagnosis
Location(s) stationed	
Report date	
Departure date	

## Case Definitions

Several CanL case definitions were used.

**CanL Case.** Military working dogs with positive CanL parasitological test results (PCR, stained smears from bone marrow, spleen, liver, lymph node, or blood).

**CanL Probable Case.** Exposed MWDs with a positive anti-CanL antibody test (ELISA, IFAT) and any clinical, laboratory, and necropsy finding, such as dry skin desquamation/scaling, facial muscle atrophy, underweight at necropsy, lymphadenopathy, conjunctivitis, or nephropathy.

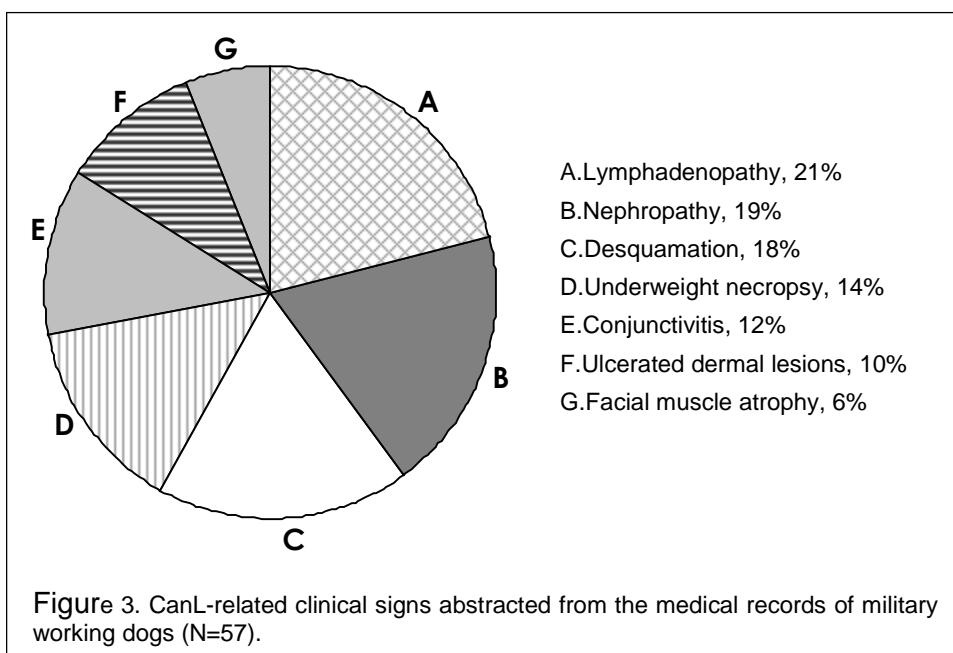
**CanL Suspect Case.** Exposed MWDs possessing any two of the previously mentioned clinical, laboratory, and necropsy findings.

## Results

No MWDs satisfied either the CanL case or CanL probable case definition criteria. Although all MWDs displayed both negative PCR and IFAT results, 16 MWDs met the CanL suspect case definition by displaying at least 2 CanL signs or symptoms after exposure to the Mediterranean Basin.

All MWDs were negative for >1:16 titer serology (N=32) and PCR results (N=36). Both negative PCR and IFAT results were obtained for 16 of 57 MWDs (28%), increasing the confidence that these MWDs were negative for CanL.

Figure 3 shows the clinical signs collated from the 57 medical records. The most frequent signs were lymphadenopathy (21%), nephropathy (19%), and desquamation (18%). Figure 4 shows the frequency of clinical signs for the number of clinical signs. Thirty-nine percent of the MWDs (N=57) had no clinical signs, while 37% had one sign, and 25% of MWDs had 2 or more clinical signs. Figure 5 shows a scatterplot of the number of clinical signs versus Mediterranean basin exposure. The Pearson correlation coefficient of -0.080 reflects the lack of association between these 2 variables (P=0.553).



## DISCUSSION

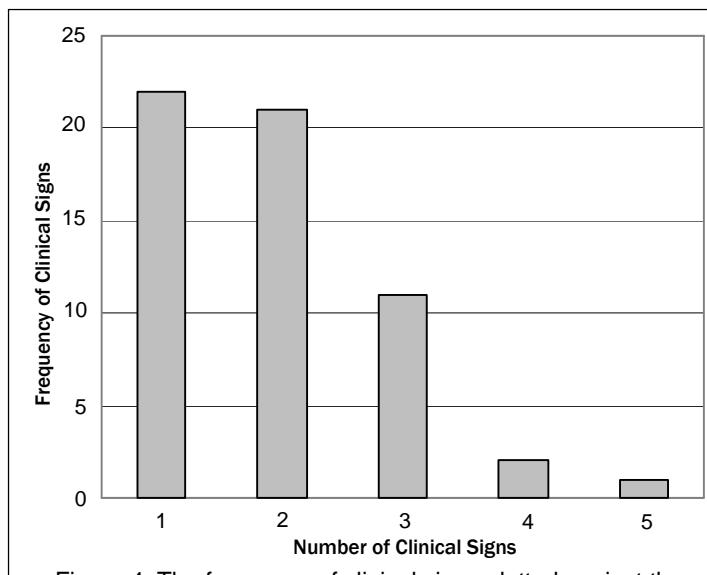
Prior to this study, the potential for MWDs to harbor subclinical CanL appeared likely, given the prevalence (10% to 37%) of CanL in southern Europe,<sup>7</sup> and the close proximity of MWDs to the competent sand fly vector. Clinical-complex masking of CanL was considered possible because of the high level of medical and nutritional support provided to MWDs. Healthy MWDs are thought less likely to display typical CanL symptoms. Stray dogs in southern Europe frequently exhibit clinical signs, largely due to immunosuppression arising from poor nutrition and inadequate medical care, including the absence of topical insecticides.

The fact that no MWDs in this study were classified as CanL cases or CanL probable cases suggests that the potential for MWDs to translocate CanL is very low. Although 16 MWDs met the CanL suspect case definition, no correlation was found between the length of time MWDs were exposed and the number of CanL-related clinical signs abstracted from medical records. However, previous studies of dogs located within the Mediterranean Basin have clearly demonstrated a positive correlation between length of time exposed and the number of dogs with CanL,<sup>23</sup> which might cast doubt on the assumption that the

abstracted clinical signs in this study are predictive for CanL.

The lack of diagnostically positive (PCR/IFAT) symptoms, or asymptomatic (based on abstracted clinical signs) MWDs in this study also suggests that MWDs are less likely to be subclinical carriers, and were probably never infected with CanL. Moreover, MWDs appear less vulnerable to the potentially infective and ubiquitous sand fly. A plausible explanation is that the regular treatment of MWDs with several topical insecticides might produce a

sand fly antifeeding effect. Recent studies support this assumption. For example, one study found that sand fly blood feeding and the survival rate of both fed and unfed flies were significantly reduced by the permethrin, deltamethrin, and fenthion treatments.<sup>24</sup> Thus, insecticides applied to MWDs for external parasite control appear to have reduced the incidence of CanL, which would, in turn, reduce the potential for MWDs to act as reservoirs for human leishmaniasis.



While the results of this study cannot definitively describe the risk of CanL to MWDs in the Mediterranean Basin, they do suggest that the potential for MWDs to translocate CanL leishmaniasis into nonendemic areas, or serve as a reservoir for human leishmaniasis, is more unlikely than previously assumed.

There are a number of limitations inherent in the design of this study. First, the selection of MWDs with Mediterranean Basin exposure could not be randomized due to the limited numbers of exposed dogs. Second, the study was descriptive in nature and provided only quantitative estimates of the magnitude of CanL infections in MWDs. The design of this prevalence study fell short of identifying causation, because the presence of CanL infections in selected MWDs could not be ruled out prior to their exposure in the Mediterranean Basin. Third, all medical record abstractions were performed after the PCR and IFAT results were collated. If any of the PCR or IFAT results had been positive, this knowledge might have biased the focus of the abstraction process. Finally, the author of this study was stationed at a veterinary clinic within the Mediterranean Basin where he diagnosed and euthanized dogs with CanL. These experiences might have affected his opinion on CanL's impact in southern Europe.

There is no 100% specific and sensitive test for CanL. The simplest and most specific diagnostic method is the demonstration of CanL amastigotes in stained smears of bone marrow or in the fine needle aspirates of lymph nodes. Utilizing these methods to find the parasite provides an extremely specific test, but the sensitivity of this approach is poor (30% with lymph node smears). For this reason, IFAT (widely considered the serology gold standard, and a 3% to 4% false-positive rate), and PCR (proven to be highly sensitive and specific) were employed. For the purpose of this study, both "possibly infected" and "highly suspected" MWDs would have been considered serologically positive. Negative IFAT results for CanL did not necessarily indicate the absence of CanL, but did indicate either the absence or the inability to detect leishmaniasis antibodies.

The causative agents of the *Trypanosoma Cruzi* and *Leishmania spp.* parasites belong to the Trypanosomatidae family and share various antigens that cause cross-reactivity in serological diagnosis. As the MWD study population might have been exposed to *Trypanosoma Cruzi*—it is endemic in south Texas, and all MWDs selected for this study spent varying lengths of time in that area—cross-reactivity concerns were addressed by using both PCR and IFAT testing. PCR tests provide greater specificity through amplification of leishmaniasis DNA. In addition, if any of the IFAT tests had been positive, serum samples would have been tested for *T. Cruzi* to quantify and control for potential cross-reactivity. Military working dogs that had a negative PCR test with both a positive CanL IFAT and *T. Cruzi* test result would have been considered cross-reactive, and not categorized as a CanL case, probable case, or suspected case.

Further research on the prevalence of CanL would be useful. Many stray dogs are found in or near US installations in southern Europe, and are often adopted by US service members and brought to the

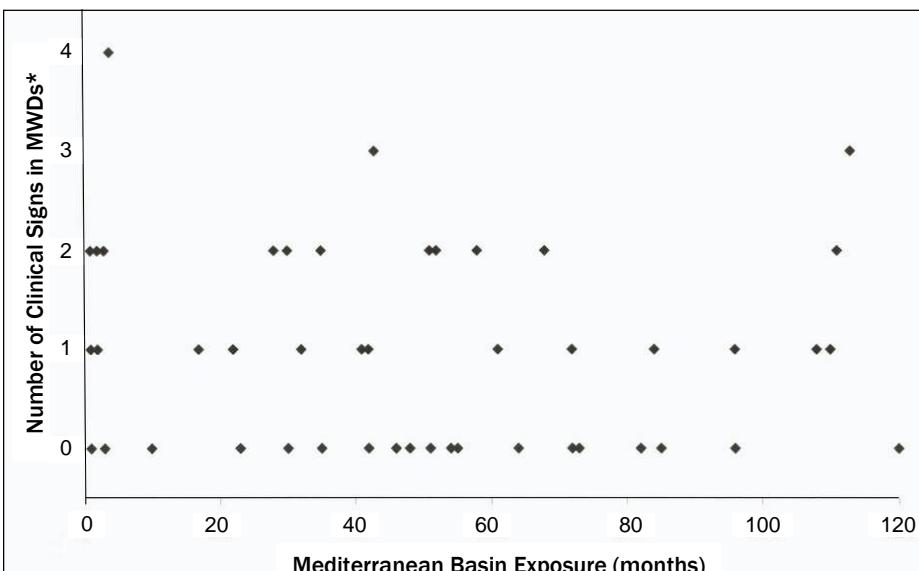


Figure 5. The scatterplot of the number of clinical signs against Mediterranean basin exposure determined in the data analysis of the military working dog\* medical records. The Pearson correlation coefficient of -0.080 reflects the lack of association between the 2 variables ( $P=0.553$ ).

## ***The Impact of Leishmaniasis on Military Working Dogs with Mediterranean Basin Exposure***

United States without being tested for CanL. According to internal US Army Veterinary Command data, approximately 1,000 privately owned dogs are imported annually from southern Europe with US military families. United States entry requirements are minimal, requiring only a current health certificate and proof of rabies vaccination. The influx of dogs and minimal screening requirements have raised concerns of importing CanL into the United States and other historically CanL nonendemic areas.

In March of 2002, the CDC, in conjunction with the US Army Veterinary Corps, started collecting serum from dogs owned by military service members returning to the United States from bases around the Mediterranean Sea. It is hoped that this project will better define the risk of importing CanL from military bases in southern Europe to nonendemic leishmaniasis areas.

### **ACKNOWLEDGEMENT**

The author thanks Dr Michelle Fleetwood at the AFIP who retrieved the formalin-fixed and paraffin-embedded liver and spleen MWD tissues for PCR testing from storage at the Walter Reed Army Institute of Research, and Lisa Hohenberg at the Walter Reed Army Medical Center for her assistance with the PCR testing.

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# ***The Hidden Work of a Laboratory Animal Veterinarian***

MAJ Craig A. Koeller, VC, USA

If one asks most people how a laboratory animal veterinarian in the Army can make a difference to the Soldiers in a time of war, the likely response will concern medical product testing, vaccines, therapies for diseases, or perhaps basic research into areas such as limb regeneration. Another possibility, but one more likely to be overlooked, is the provision of help in the training of medical personnel, especially the combat medic. This training will likely be multifaceted, but may involve the use of animals, thus requiring a veterinarian to oversee their use. The final training is important and can yield great results,<sup>1</sup> but how is such training developed and what occurs in the hours behind the visible portion of running such training? This article provides an inside look from the perspective of a veterinarian, based on my experience in assisting in the implementation of a combat trauma course at the Madigan Army Medical Center.

The effort started in the most basic fashion, with someone perceiving a need for Soldiers (in this case, combat medics) to get more trauma training to help save the lives of their comrades on the battlefield. In my specific circumstance, it was a chance lunchtime meeting with one of the local brigade surgeons who asked if I knew of a group in the hospital that could provide such training. The entire group must be willing to support the development and execution of the training. The group must determine exactly what subjects should be taught, relevance of subjects trained as related to injuries encountered, and actual method of providing the training. The subjects taught and relevance are mainly decided by the doctors for whom the medics work and the surgeons who see the results of the treatments medics provide.

Medical simulators, didactics, skill stations, and animal models all have a role in this training. The US Animal Welfare Act<sup>2</sup> and *Army Regulation 40-33*<sup>3</sup> require that the veterinarian be intimately involved in all aspects of animal use, to include helping the responsible individual write the proposal for the training program. The veterinarian must act as the advocate of the animal in trying to make sure the

appropriate species will be used, only the actual number of animals that are needed will be used, and that the planned and performed procedures match after the protocol has been approved. This information is written into a proposal called an animal protocol that must be approved by an Institutional Animal Care and Use Committee (IACUC). This committee will review the proposal to determine if

1. the proposal warrants using animals,
2. the procedures to be performed are appropriate to the study or training goals,
3. the personnel that will be conducting the training are qualified to do so,
4. there are alternatives to using animals (at least to some extent by utilizing medical simulators), and
5. the welfare of the animals is protected.

The Committee can accept the proposal, require revisions before accepting the proposal, or withhold approval. In the case of trauma training, the discussion on such a protocol will tend to be quite involved. Items of interest normally include:

- Appropriate anesthesia will be maintained when animals are being used.
- The animal will be euthanized in a humane manner.
- Maximum use of the animal.
- Training of the medics will be balanced.
- Procedures to be performed match with the people who will perform them.

Anesthesia is simpler to maintain when the animal is in a surgery type setting where gas anesthesia can be used, but more difficult in a field environment. The “casualty” will be moved and transported in a field environment, and injectable anesthesia is used. In the field, the veterinarian and veterinary staff must be more vigilant in constantly assessing the animal’s

plane of anesthesia as each animal will metabolize the injectable anesthetic at a different rate.

After the local IACUC approves the protocol, the protocol is submitted to the Clinical Investigations Regulatory Office (CIRO) to ensure that all applicable laws and regulations will be followed. After reviewing the protocol, CIRO will address any concerns they have to the local IACUC.

There are 2 other areas within which the veterinarian must also interact in order to make a course such as this possible. Public affairs officials must know about the course and be fully briefed on what will be occurring and how it is conducted. It is especially important that it is well understood that the animals will be anesthetized and monitored at all times to ensure they feel no pain. Photographs taken of animals used during this training could result in a large outcry from animal rights proponents, especially if misinformation accompanies the pictures, such as no acknowledgement that the animals are anesthetized. It is the job of public affairs officials to help diffuse the misconceptions and ensure facts are disseminated if information must be provided to outside media or in response to questions from individuals.

The last agency that should be consulted is office of Occupational Health and Safety. Any work around animals carries some risks. The most common risk is the allergens that animals carry. People can have an allergic reaction to the fur, dander, or other aspects of the animals. Occupational Health will help to perform a risk assessment for exposures to potential allergens for both participants and instructors. In cooperation with Occupational Health, part of the veterinarian's briefing about the lab and field phases includes information about possible zoonotic diseases the animals may harbor, and as well as the potential allergens and allergies. Students are warned about the possibility of allergies. They are asked to inform the instructors or Occupational Health if they suffer from asthma or known allergies that could affect their health during the course.

The veterinarian oversees the purchase and care of all animals. The veterinary staff must anesthetize and prepare the animals for the controlled skills laboratory and the field exercise. While the instructors demonstrate skills or assess the skills of the students,

the veterinary staff works in the background controlling the animal's anesthesia to ensure that no pain is felt.

It is extremely satisfying to be a participant in a course such as the Madigan combat trauma course, as the growth of the medics' skills is clearly evident as the course progresses. Their confidence in their skills increases as well. The hours can be long, especially when performing field training. The US military owns the night, therefore medics must treat patients in the dark. We honor the creed "to train as we fight," accordingly, the trauma training of medics must include training during the hours of darkness. Thus, the final day of the field phase portion of the Madigan combat trauma course would last from about 9 AM until midnight.

The laboratory animal veterinarian is a vital member of any training involving animals. The person filling this role is not only in a highly visible position in the conduct of the actual training, but is also very much involved in the work required to obtain approval for such a program. Indeed, perhaps the most important role of the veterinarian is helping to obtain protocol approval, and ensuring that all of the requirements of federal law and military regulations are followed. Although it may not be as visible or appreciated, it is this work that makes the training possible.

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# ***Challenges in Biodefense Research and the Role of US Army Veterinary Pathologists***

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## **INTRODUCTION**

The use of infectious agents as biological weapons dates at least as far back as the 14th century. Since that time, there have been documented instances of the deliberate use of biowarfare agents such as plague, smallpox, glanders, and anthrax to achieve military goals.<sup>1</sup> Extensive state-sponsored biological weapons programs were conducted in the 20th century by Germany, Japan, and the former Soviet Union.<sup>2</sup> The United States also operated a program that included the weaponization of several infectious agents until 1969, when President Richard Nixon ended the country's offensive weapons program. Since that time, the United States has limited its efforts to defensive countermeasures against biological agents. Concerns about the threats posed by biological weapons have intensified in recent years due to information brought to light since the fall of the Soviet Union, attempts by terrorist groups and others to obtain biowarfare agents, and as a result of the anthrax murders in the United States in 2001. Concern about biological weapons is further increased because of an understanding of the potential harm presented by genetically engineered agents. Not surprisingly then, the matter of biological weapons is of great interest to the Department of Defense (DoD). The infectious agents typically associated with biological weapons are also of concern because military members may be exposed to them naturally in areas of military deployments. The nature of the disease that may result from exposure of troops to biowarfare agents, whether naturally or through deliberate spread, can vary greatly. Some biowarfare agents cause incapacitating disease with high morbidity, while others can be highly lethal. Either way, they pose a major challenge for combat commanders and support personnel, especially medical units. There is, therefore, a great need to develop effective countermeasures to contend with the threat posed by biological agents.

Countermeasures against biological agents include diagnostics, vaccines, therapeutic agents, and operational practices. Historically, much of the research necessary to develop such countermeasures has fallen upon the military because it was not considered to have much relevance to the civilian population. That philosophy has changed in recent years, however, especially in light of the anthrax murders. Nonetheless, the military has played a lead role in the nation's biodefense research program. The US Army Medical Research and Materiel Command is the executive or lead agency responsible for 2 key biodefense programs, the Medical Chemical and Biological Defense Research Program and the Military Infectious Diseases Research Program. Actual research investigations under these programs are performed at a variety of DoD and civilian institutions. The majority of military studies that require biological containment are performed at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), which houses biocontainment facilities at both biosafety level 3 (BSL-3) and BSL-4 (maximum biocontainment). Research at USAMRIID is conducted in compliance with the Animal Welfare Act\* and other federal statutes and regulations relating to animals and experiments involving animals, and adheres to principles established by the Institute of Laboratory Animal Research.<sup>3</sup> The facility where research is conducted at USAMRIID is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (5283 Corporate Drive, Suite 203, Frederick, MD 21703-2879).

The challenges to developing countermeasures to biological agents are many and varied. The development of vaccines, antibiotics, and other therapeutics for use in humans is a process that, under

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\*7 USC, 2131-2159. Available at: <http://www.nal.usda.gov/awic/legislat/awa.htm>.

the best of circumstances, takes several years and costs millions of dollars for each product. Product development typically requires advanced knowledge about the pathogenesis of the disease agent in humans. It also requires that one or more appropriate animal models exist for which the disease course is sufficiently similar to the human condition, so that the safety and efficacy of the vaccine or therapeutic agent can adequately be assessed. For many biowarfare agents, the efficacy of vaccines or therapeutics cannot even be tested in humans, for ethical or other reasons. This has recently led to the acceptance of the so-called “animal rule,” which permits the Food and Drug Administration (FDA) to rely on evidence from animal studies to judge the likely effectiveness of vaccines or therapeutics in humans. This approach requires that the pathogenesis of a particular disease agent is well demonstrated in one or more animal models and that the nature by which a vaccine or therapeutic would provide protection is well understood. The use of the animal rule to facilitate development of biowarfare countermeasures provides a key tool in biodefense programs, however, it places even greater emphasis on the proper conduct of animal studies, including pathogenesis studies and vaccine or drug therapy studies involving pathology.

Army Veterinary Corps (VC) officers are key components of the research, support, and headquarters staffs of USAMRIID. Several VC officers have served as USAMRIID commanders and deputy commanders in recent years. Veterinary Corps pathologists direct USAMRIID’s pathology services, perform all phases of pathology analysis for institute studies, and conduct primary research. Veterinary Corps pathologists represent a very small proportion of the officers in the Army Medical Department, yet they are some of the most highly trained professionals in the US Army and perform some of the most demanding and potentially hazardous portions of DoD biomedical research. Pathology training of graduate veterinarians in the Veterinary Corps consists of a rigorous residency program at the Armed Forces Institute of Pathology (AFIP), leading to board certification. Upon completion of the residency, veterinary pathologists (area of concentration 64D) are assigned to Army, Navy, Air Force, and joint laboratories at a number of sites, both in and outside of the United States. These include the AFIP, Walter Reed Army Institute of Research, US Army Medical Research Institute of Chemical Defense, Air Force Research Laboratory, and Armed Forces Research Institute of Medical

Sciences. Some VC pathologists later enter graduate programs leading to the PhD degree. Areas of biomedical research in which veterinary pathologists are involved range from combat casualty care to chemical agent countermeasures to studies of a variety of infectious diseases. These research areas rely heavily on animal models as surrogates for human conditions. Pathologists at USAMRIID specifically study countermeasures to some of the most lethal biological agents known, including plague, anthrax, botulinum neurotoxin, Ebola virus, and smallpox.<sup>4</sup> Working with these agents requires that veterinary pathologists at USAMRIID conduct research in BSL-3 and BSL-4 laboratories. Pathologists may gain sufficient experience and knowledge to become subject matter experts on particular agents, and are often called upon to provide their expertise to organizations within and external to the military. For example, Army pathologists from USAMRIID have played major diagnostic roles in disease outbreaks, such as the importation into the United States of Ebola virus in monkeys, the emergence of West Nile virus in birds and horses, and an outbreak of monkeypox in imported pet animals.<sup>5-8</sup> Also, USAMRIID pathologists have participated in field studies around



USAMRIID pathology personnel at work in a biosafety-level-4 laboratory. Biocontainment and personal safety considerations for research with certain pathogens mandate a variety of preventive measures, including that personnel wear self-contained, chemically disinfectable suits supplied with HEPA-filtered air.

## Challenges in Biodefense Research and the Role of US Army Veterinary Pathologists

the world related to diseases like tularemia, plague, and Ebola virus. Such studies are either DoD sponsored or pathologists may serve as temporary consultants to organizations like the World Health Organization. The role that USAMRIID pathologists play in biodefense research has even been featured in the popular literature.<sup>9,10</sup>



A USAMRIID pathologist (facing) processing samples in a jungle laboratory. USAMRIID pathologists have deployed in a variety of scientific roles, including investigations to discover the natural reservoir of Ebola virus in the Democratic Republic of the Congo and the Ivory Coast (shown above).

In the remainder of this article, we illustrate important challenges in biodefense research by considering 3 important biowarfare-related diseases of concern to the DoD. We discuss the nature of the challenge presented by each disease, review key biological features of each, and highlight the role of army veterinary pathologists in biomedical research through their contributions to the knowledge base of these disease areas.

### VENEZUELAN EQUINE ENCEPHALITIS

Venezuelan equine encephalitis (VEE) is an important mosquito-transmitted natural disease of horses. It is similar to 2 other members of the *Alphavirus* genus, eastern equine encephalitis and western equine encephalitis. Despite their names, however, all 3 viruses are significant natural causes of human disease, are considered biowarfare agents, and have been the subject of extensive vaccine development efforts at USAMRIID. Investigations with these viruses are conducted at BSL-3. In humans, VEE virus usually causes an acute, febrile, incapacitating disease. On

occasion, VEE causes large outbreaks, such as the 1995 epidemic in Columbia and Venezuela that infected as many as 100,000 people.<sup>11</sup> The virus is also highly infectious by aerosol, having caused at least 150 infections in lab workers, most of which were probably the result of aerosol infection.<sup>12</sup> The VEE virus is also easily grown to high titer in culture and is relatively stable in storage, conditions that facilitate weaponization. Although VEE is rarely lethal in adults, the virus could serve as a significant incapacitating agent if used in a biological attack. The VEE vaccine program at USAMRIID has been active for several years, but efforts have been confounded by 2 important factors. One is the need for a vaccine that demonstrates effective immunity against aerosol infection, a more difficult standard to achieve than immunity against natural infection by mosquitoes. Further, multiple serotypes of VEE viruses exist and the virus is readily amenable to genetic manipulation, so a vaccine must be capable of providing a significant degree of cross-protection against a number of virus strains.

From investigations of VEE outbreaks together with experimental animal studies, there is a fair amount known about the pathogenesis of VEE. The incubation period of VEE virus in humans is about 1 to 4 days, after which patients develop fever, severe headache, myalgia, and chills, lasting from a few days to 2 weeks.<sup>11,13,14</sup> Infection of the central nervous system develops in a minority of VEE cases, resulting in convulsions, paralysis, and sometimes death. A number of animal species are susceptible to VEE virus and they mimic important aspects of the human disease.<sup>15</sup> Rhesus and cynomolgus macaques are both susceptible to infection with VEE virus and exhibit disease much like that of humans.<sup>16</sup> Their usefulness is limited though by their cost, availability, size, and animal-use concerns. Therefore, mice have been the most extensively used animal model for VEE. Like humans, mice also exhibit a biphasic illness with infection of peripheral tissues preceding neuroinvasion. In mice, though, 100% of susceptible strains develop central nervous system infection and die.

In the mouse model of VEE that mimics mosquito transmission of virus, so-called dendritic cells in the skin are the first type of cell infected.<sup>17</sup> The mobile dendritic cells transport virus to the draining lymph

node, where initial replication occurs.<sup>18</sup> This results in viremia, after which virions in the blood appear to gain access to the brain by way of the olfactory nerves.<sup>19</sup> Virus then spreads throughout the brain and into the spinal cord.<sup>20,21</sup> Neurons are the primary target of viral infection in the brain, and they suffer massive cell death.<sup>20-22</sup> At USAMRIID, aerosol studies of VEE in mice have been used to study the course of disease expected in a biowarfare event. By the aerosol route, VEE virus first infects the olfactory neurons of the nasal tract.<sup>20,21</sup> Because the olfactory neurons project axons directly to the brain, VEE virus rapidly invades the brain by this route of inoculation, independent of the development of viremia. Although other tissues are also infected after aerosol administration, the rapid and overwhelming infection of the brain is the key event. By comparison, macaques infected with VEE virus by both peripheral and aerosol routes develop fever, viremia, lymphopenia, and occasionally encephalitis, but rarely die.<sup>16</sup>

Neuroinvasion by VEE virus independent of viremia illustrates a key challenge in developing vaccines effective against aerosolized virus. Viremia appears to be an obligatory step in the pathogenesis of natural VEE infection, therefore the development of serum neutralizing antibodies (IgG) protects against a peripheral infection, as is also the case for other alphaviruses.<sup>23,24</sup> However, neutralizing IgA antibody, not serum IgG antibody, is required to protect mice against aerosol challenge with VEE virus.<sup>23,25</sup>

An effective VEE vaccine must therefore be able to stimulate sufficient mucosal IgA antibodies to protect against aerosol infection of the nasal tract, as would be expected in a biowarfare event.

TC-83, a live-attenuated VEE vaccine developed at USAMRIID, is used to vaccinate laboratory workers who handle VEE virus under Investigational New Drug status. TC-83 is limited in its broader usefulness, though, because it causes adverse side effects in approximately 23% of human recipients, an additional 18% of recipients fail to develop protective serum antibodies, and the vaccine does not provide sufficient protection against aerosol infection in animals.<sup>23,25</sup> V3526 is a rationally designed, genetically engineered VEE vaccine, under development for several years.<sup>26</sup> Studies at USAMRIID comparing V3526 and TC-83 showed that V3526 induced protective serum and mucosal antibody titers with fewer nonresponders in

mice and demonstrated better protection against aerosol infection of mice and nonhuman primates.<sup>25,27</sup> It also protected mice and nonhuman primates against challenge with a heterologous strain of VEE virus.<sup>28,29</sup> Additional USAMRIID studies showed that V3526 appeared safer than TC-83 in rodents, being less reactogenic and exhibiting significantly reduced neurovirulence and decreased reversion potential.<sup>20,25,28</sup> By several measures based on animal studies, V3526 appears to be a safer and more effective vaccine candidate than TC-83.<sup>16,30</sup> Completion of human trials with V3526 required for FDA approval and licensure remains a significant hurdle before this vaccine can be used in its target population.

## SMALLPOX

Smallpox virus is an agent of potentially devastating impact to both the military and the civilian communities. The virus is easily transmitted from person to person and produces disease with a mortality rate around 30%.<sup>31</sup> For centuries the scourge of mankind, smallpox was officially considered eradicated in 1980, providing perhaps the greatest public health achievement against an infectious disease in history. Official repositories of variola virus, the agent of smallpox, continue to exist; however, they are confined to biocontainment facilities at the Centers for Disease Control and Prevention (CDC) in Atlanta, GA, and at the State Research Centre of Virology and Biotechnology in Russia.<sup>32</sup> Additional clandestine sources may also continue to exist in other locations around the world.<sup>33</sup> The Soviet Union is reported to have manufactured large quantities of variola virus for military purposes.<sup>1,34</sup> Variola virus is considered relatively stable in aerosols, making it amenable to weaponization. For these reasons, smallpox remains a serious concern as an agent of biowarfare or bioterrorism. This concern is made greater because population immunity to variola virus has greatly waned since active vaccination of people ceased after the eradication of smallpox. Efforts to reconstitute the smallpox vaccination program for individuals considered at risk have been met with resistance, due to concerns about the adverse events that may be associated with the vaccine. Complications such as postvaccinal encephalitis and generalized vaccinia are a particular risk for people with immunosuppression or existing skin disease such as eczema.<sup>33</sup> For various reasons then, any deliberate use of smallpox virus

would place great demands on our abilities to quickly detect its introduction, to treat affected individuals, and to limit its spread.

Animal models of smallpox are therefore needed to develop improved countermeasures like antiviral drugs, an effective and safe vaccine, and rapid diagnostic tests. In addition, there is a need to better understand many of the basic pathophysiological and immunological aspects of smallpox in humans, as the disease was eradicated before many modern scientific techniques could be applied to human infections.<sup>35,36</sup> Variola virus is one of 5 species in the genus *Orthopoxvirus* that may cause active infection in humans. The others are monkeypox virus, cowpox virus, and to a lesser extent, camelpox and vaccinia viruses, the latter being the virus used for smallpox vaccination. Additional orthopoxviruses, such as rabbitpox and ectromelia virus (mousepox), are not pathogenic for humans, yet like the other orthopoxviruses, they can serve as useful animal models for understanding smallpox. While monkeypox, cowpox, and camelpox are zoonotic diseases, variola virus was strictly a human pathogen in nature. In fact, the eradication of smallpox from the world was successful much due to the fact that animal reservoirs of the virus did not exist to reintroduce the virus into the human population. The development of an animal model for smallpox research is a very difficult endeavor because of the resistance of animals to infection, a challenge made even greater by the logistical complications of working with variola virus. Not only is variola virus a BSL-4 agent, but laboratory work with the virus in the United States can only be performed under maximum biocontainment at the CDC. To meet these challenges, research at USAMRIID makes use of a variety of animal models of orthopox disease aimed at fulfilling the animal rule.

There are 2 varieties of smallpox, variola major and variola minor, based on severity of disease and mortality rate. Variola major has a case-fatality rate of 30% among unvaccinated persons, whereas variola minor has a 1% case-fatality rate. Factors that affect mortality include age, viral strain, immune response, and nutritional status. Both humoral and cell-mediated immunity are important in recovery from this disease.<sup>31</sup> The classic, or ordinary, form of smallpox is a febrile disease with a characteristic vesiculopustular skin rash. It is naturally spread through aerosol or

droplets from oropharyngeal secretions, or by direct contact with infected persons or fomites.<sup>37</sup> The cause of death from smallpox appears to be the result of a cytokine storm, a host response to viral infection previously referred to as “toxemia,” as well as by direct virus tissue damage.<sup>35,37,38</sup> Other clinical forms of smallpox, such as the hemorrhagic and flat-type, are less common but highly pathogenic. USAMRIID investigators and pathologists working at the CDC have recently established a nonhuman primate model of smallpox by infecting cynomolgus macaques (*Macaca fascicularis*) with strains of variola intravenously. Using high doses of virus, monkeys develop systemic disease that closely resembles the hemorrhagic form of human smallpox, and exhibit uniform lethality.<sup>38</sup> Pathology demonstrated viral infection and organ dysfunction affecting the lymphoid tissues, skin, oral mucosa, gastrointestinal tract, reproductive system, and liver. Elaboration of cytokines was also shown in these monkeys. Inoculation of cynomolgus monkeys with high doses of variola by aerosol produces a nonlethal illness in which the monkeys develop a mild skin eruption but do not develop lesions typical of classic smallpox.<sup>39</sup> The nonhuman primate model of variola infection has already been used to evaluate the efficacy of the antiviral drug cidofovir, showing that the drug can significantly lower viremias and the development of skin lesions, as well as prevent death.<sup>35</sup>

Monkeypox is not only a very useful animal model for smallpox, it is sometimes a fatal human pathogen as well. Several human cases of monkeypox occurred in the United States in 2003 after introduction of the virus by imported rodents from Ghana.<sup>6</sup> Monkeypox is generally very similar clinically to smallpox but the virus does not spread as easily among humans, likely decreasing its effectiveness as a biowarfare agent. The route of infection by monkeypox virus is also similar to that of smallpox, including by the respiratory route.<sup>31</sup> A significant benefit of the monkeypox model is that experiments can be conducted under BSL-3 at USAMRIID. In particular, the aerosol model of monkeypox is relevant to the disease expected after a biowarfare release. A key USAMRIID pathology study showed that aerosol infection of cynomolgus monkeys produced lethal multisystemic disease and that viral infection of the lower airways causing bronchopneumonia was prominent.<sup>40</sup> Monkeys have also been shown to be susceptible to monkeypox virus

by the intravenous (IV) route.<sup>35</sup> The IV and aerosol models of monkeypox have both been used to investigate alternative methods of vaccination, with promising results.<sup>35</sup> The IV monkeypox model has been used to show the efficacy of cidofovir treatment, similar to the smallpox model.

A variety of additional animal models, including infection of mice with ectromelia and vaccinia viruses, have been used to study mechanisms of orthopoxvirus infection. Another USAMRIID pathology study relevant to biowarfare concerns showed that infecting mice with aerosolized cowpox virus reproduced key features of orthopoxvirus disease and specifically targeted the respiratory system.<sup>41</sup> This model was used to show that aerosolized cidofovir administration could provide a useful therapy for aerosol infections by smallpox and monkeypox while limiting some of the potential toxic side effects of this drug.<sup>42</sup> Many challenges remain in the use of animal models to develop countermeasures to smallpox, including a more thorough understanding of poxvirus virulence factors, host-pathogen interactions, and the basic pathophysiology of infection. In particular, it remains to be determined which of the monkey models, monkeypox or variola, better recapitulate smallpox infection in humans and how appropriate either of these models is to infection with smallpox virus in the event of a biowarfare release or the subsequent human to human transmission that might follow a biowarfare release.

## EBOLA VIRUS

Ebola hemorrhagic fever is one of the most lethal diseases of humans, with mortality rates in natural outbreaks approaching 90%.<sup>43,44</sup> A number of confirmed outbreaks of Ebola virus (EBOV) have been documented since the virus was first recognized in 1976, most of these arising in central Africa. Human outbreaks appear to be initiated through the handling of infected wild animals, in particular chimpanzees and gorillas.<sup>45,46</sup> Subsequent human to human transmission then occurs through contact with infected bodily fluids, secretions or tissues, usually among family members or from infected patients to medical personnel. With regard to the potential for weaponization and biowarfare use, EBOV is fairly stable under harsh environmental conditions and is highly infectious and stable as fine aerosols.<sup>44</sup> Ebola virus is a BSL-4 pathogen.

The incubation period of Ebola virus infection in humans is relatively broad at 2 to 21 days, however, most cases probably involve a much shorter incubation period with death around 7 to 10 days after infection. Infection is characterized initially as an acute, severe, febrile illness with evidence of vascular involvement.<sup>47,48</sup> Later in the disease course, immune suppression, multisystem dysfunction, shock, and coagulopathy occur. Patients often begin to develop disseminated intravascular coagulopathy (DIC) by day 5 postinfection. The key targets of Ebola virus are cells of the mononuclear phagocyte system, hepatocytes, and possibly endothelial cells.

Pathologists at USAMRIID have worked extensively with other investigators to characterize animal models of Ebola virus to begin to understand its extreme virulence. To date, mouse, hamster, guinea pig, and nonhuman primate models of Ebola virus infection have been developed and used to investigate a variety of pathogenetic factors that contribute to virulence. As is the case with both VEE virus and smallpox, the nonhuman primate model of Ebola virus infection more closely resembles the human condition. Nonhuman primate studies have shown that Ebola virus can be transmitted by a variety of routes, including intramuscular inoculation, aerosol administration, and by the oral and conjunctival routes.<sup>18</sup> Studies of mice, guinea pigs, and nonhuman primates have all shown that monocytes and macrophages together represent an early and sustained target of EBOV and are the main means by which the virus is disseminated throughout the body.<sup>18,49-52</sup> These findings help confirm the importance of infected macrophages in human Ebola hemorrhagic fever. Dendritic cells in animals appear to be key early targets as well.<sup>53</sup> Ebola virus also targets and causes extensive damage to hepatocytes, adrenal cells, fibroblasts, and a variety of epithelial cell types later in the course of disease. Lymphoid damage and thrombocytopenia are also prominent features of Ebola virus infection, but these are indirect effects of viral infection. While endothelial cells have been considered important in the pathogenesis of human Ebola hemorrhagic fever, the animal studies have questioned this paradigm, suggesting that the hemorrhagic manifestation of Ebola hemorrhagic fever is more likely the result of a cytokine storm subsequent to infection of mononuclear phagocytes than a direct effect of virus-induced cytolysis of

endothelial cells.<sup>49,53</sup> Virus-mediated alteration of mononuclear phagocytes and dendritic cells also appears critical to the immune system disruption that is another key feature of Ebola infection.

While the mouse and guinea pig models share the key features of Ebola virus infection with respect to viral tropism, organ damage, and lethal disease course, they do not exhibit other important features of the disease. The mouse model of Ebola virus characteristically lacks tissue fibrin deposition and DIC, and infected mice fail to develop the characteristic maculopapular rash or overt hemorrhage seen in human and nonhuman primate Ebola virus infections.<sup>51</sup> Experimentally infected guinea pigs exhibit variable tissue fibrin deposition and only limited hemorrhagic lesions.<sup>49</sup> Nonhuman primates, however, develop fibrin deposition, DIC, and hemorrhage similar to humans. Thus, nonhuman primates exhibit a variety of the features of Ebola hemorrhagic fever seen in humans and generally constitute a more relevant animal model.

One of the important benefits of pathogenesis studies is that they can identify avenues to develop therapeutic countermeasures. Therefore, the various animal models of Ebola hemorrhagic fever continue to be used to explore the molecular mechanisms that underlie the severe nature of this disease. As a direct result of such research, the development of a potential therapy to mitigate coagulopathy by treatment with a recombinant inhibitor of tissue factor VIIA has shown promise in nonhuman primates infected with Ebola virus at USAMRIID.<sup>54</sup> A variety of other mediators of coagulopathy and inflammation remain to be explored. In addition, animal models of Ebola virus have been extensively utilized in vaccine development. The development of a safe and effective Ebola virus vaccine represents another difficult challenge of biodefense research. Inactivated vaccines have not shown much promise in animal studies.<sup>55,56</sup> Attenuated vaccines for an agent like Ebola virus make poor candidates because of concerns over reversion to virulence. As a result of these concerns, a number of novel alternative methods have been recently studied, with varying success in animals. These include DNA vaccines, virus-like particle vaccines, and vesicular stomatitis virus-based vaccines.<sup>56</sup> Recently, a DNA vaccine for Ebola virus was shown safe and immunogenic in human testing.<sup>57</sup> Many hurdles remain

in the search for effective Ebola virus vaccines and therapies, and ultimately the testing of such countermeasures for efficacy will require the use of appropriate animal models in order to fulfill the FDA's "animal rule."

## **SUMMARY**

For years the nation's development of medical countermeasures to biowarfare agents has primarily existed as the domain of the United States military, but it has taken on increased urgency in the last few years. The realization that the civilian population is also at risk from biological agents has resulted in the institution of new biodefense programs at a variety of nonmilitary organizations. USAMRIID, a long-time leader in the nation's biodefense effort, will soon be joined by other US government agencies as part of a planned National Interagency Biodefense Campus at Fort Detrick Maryland.\* US Army veterinary pathologists at USAMRIID have played an important role in the nation's biodefense effort, along with our veterinary colleagues representing other specialties, our military colleagues in other Army Medical Department corps, and our civilian colleagues. Together, we will continue to strive to develop the diagnostics, vaccines, therapeutic agents, and operational practices that are required to meet the great demands posed by the threat of biowarfare agents.

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\*Information about the National Interagency Biodefense Campus is available at <http://www.detrick.army.mil/nibc/nibc01.cfm>.

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# A Veterinary Comparative Medicine Officer's Dream Assignment

MAJ Sam Yingst, VC, USA

## ABSTRACT

The 5 Department of Defense (DoD) overseas laboratories conduct research on a vast array of infectious diseases.<sup>1,2</sup> This commentary focuses on the role of the Naval Medical Research Unit No. 3 (NAMRU-3, Cairo, Egypt) in influenza surveillance and research, with emphasis on the role of the comparative medicine (US Army military occupational specialty 64E) veterinarian assigned there. Every year, tens of thousands of members of the US Armed Forces are vaccinated for so-called “seasonal influenza.” The vaccine used is reformulated annually, based on antigenic characterization of viral isolates generated through global surveillance. NAMRU-3 contributes to this global surveillance for the Eastern Mediterranean region. The emergence of H5N1 Highly-pathogenic Avian Influenza (HPAI) and concerns over its possible role in precipitating a pandemic have accentuated the role of veterinarians with field and laboratory diagnostics experience in this system.

## BACKGROUND

The World Health Organization (WHO) global influenza surveillance system is comprised of approximately 110 National Influenza Centers (NIC) and 4 WHO Collaborating Centers (WHOCC) for Influenza. The 4 WHOCCs are located in the United Kingdom,\* Japan,† Australia,‡ and the United States (CDC§). The intent of the system is that NICs take samples from a cross section of the population suffering from “influenza-like illness,” isolate viruses, conduct initial subtyping, and forward representative isolates—especially any that cannot be subtyped—to a WHOCC. For much of the world, “ordinary” seasonal influenza is not seen as a public health priority, so internal funding is not available. The currently recognized NICs throughout the world are shown in the Figure. NAMRU-3 is situated within one of the largest regional gaps in this surveillance network. NAMRU-3 is now funded by CDC, the DoD Global Emerging Infections Surveillance and Response System, and others to enhance NIC productivity in its region. These agencies fund NAMRU-3 because of the facility’s inherent laboratory capacity (including a biosafety level [BL] 3 that can be used as a BL4 in need), strategic location, and, most important, its demonstrated ability to build international

relationships and, through these, to enhance host country laboratory, surveillance system, and research capacity. NAMRU-3 has a long history of working with zoonotic disease, and an equally long history of including veterinarians in its public health team, a critical reason that the institution is capable of responding to emerging disease threats such as avian influenza.

The force protection benefit of enhanced influenza surveillance is twofold:

1. Greater refinement of information leading to the biannual vaccine decision such that the vaccine used is more optimal.
2. Actionable information about novel strains circulating in regions in which troops are deployed.

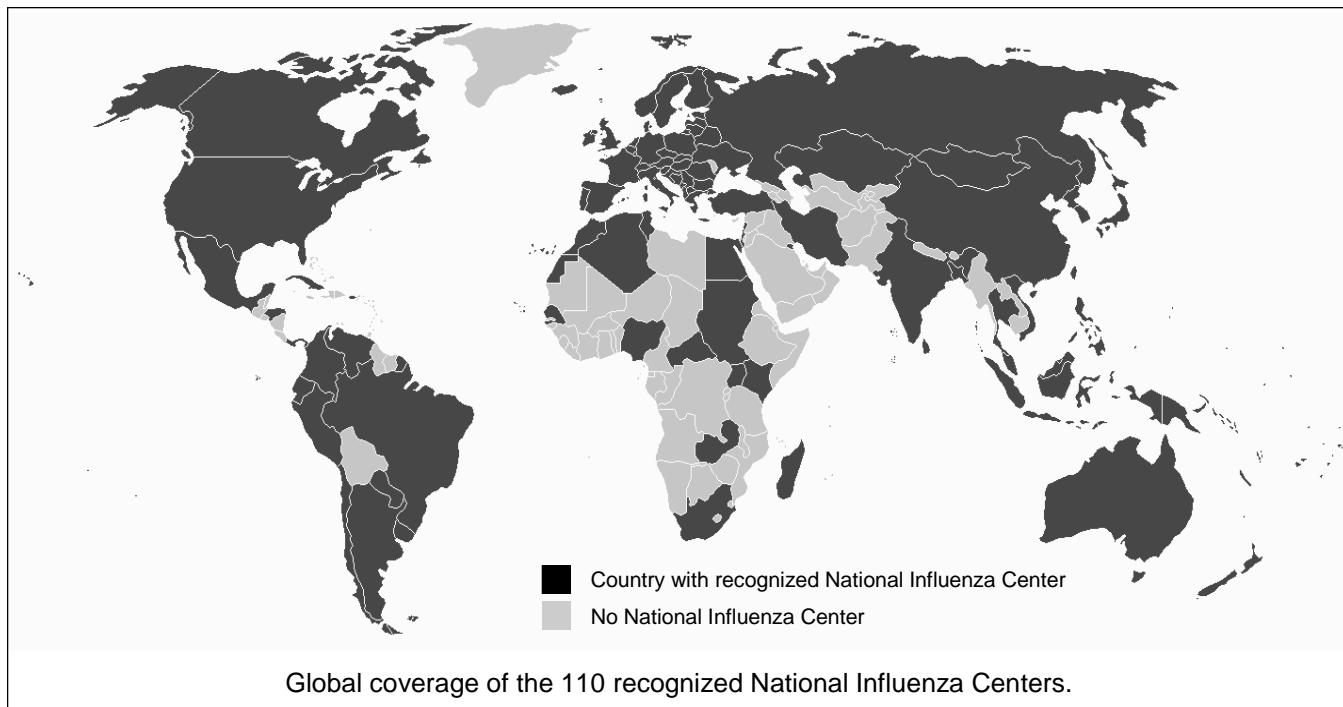
Currently, donors and even public health professionals frequently talk about avian influenza (AI) as a human disease. Indeed, there have been over 300 cases of influenza in humans caused by H5N1, and over half have died, but it is eminently clear that the virus in its current form requires a very high dose to infect humans and remains first and foremost a disease of (primarily gallinaceous) poultry. However, about 30,000 people die each year in the United States from seasonal influenza, and the world wide annual death toll from seasonal influenza may range into the hundreds of thousands. A pandemic virus is widely viewed as having the potential to kill millions, and this

\*National Institute for Medical Research (London)

†National Institute of Infectious Diseases (Tokyo)

‡Centre for Reference and Research on Influenza (Victoria)

§Centers for Disease Control and Prevention



is the valid basis for concern about the continued circulation of H5N1 viruses. Approximately a billion birds have been affected by H5N1 and the virus probably replicates trillions of times in each infected animal. A simple mutation could lead toward increased human to human transmissibility, and also raise the chances for a reassortment\* event in some animal that is coinfecte

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diagnose acute cases in avians or humans. Testing with PCR requires extensive training, experience, and consultation, but we have shown that these are not insurmountable obstacles, even in Afghanistan despite the ongoing conflict. Such places cannot be neglected because they are difficult. On the contrary, this difficulty should stimulate even greater emphasis, but to meet that need sometimes incurs significant risk. Such an effort is therefore the clear purview of uniformed laboratory diagnostic specialists. The US Army comparative medicine veterinarian is uniquely qualified to fulfill all aspects of this need.

It should be obvious that this work is also a critical element in our effort to maintain the image of the United States as a partner in peace. NAMRU-3 acknowledges that a major impetus for our work is to serve as an advocate of US foreign policy, not necessarily in a purely scientific capacity.

While the conversation among laypeople about AI, pandemic influenza, and seasonal influenza often becomes clouded, the current situation in which H5N1 HPAI is viewed as the greatest threat for precipitating a pandemic in humans is beneficial in terms of providing impetus for funding that can then be used more broadly. Constant effort is required to keep the focus on the fact that currently this is fundamentally a disease of animals, and its greatest impact is on nutrition and income of poultry owners, not directly on their health.<sup>4</sup> This is another reason that it is a

\*The mix of 2 different influenza viruses

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fortunate time for veterinary public health professionals. Leaders at all levels now recognize the importance of having a multidisciplinary team to tackle this multifaceted issue. This has allowed veterinarians, especially those with laboratory diagnostics skills, to serve in a unique capacity as medical diplomats.

### **APPROACH**

Because the military staff of the overseas laboratories occupy a position that would otherwise be available for an additional, currently much-needed line Soldier or Sailor, and because the research and surveillance role is widely seen as similar or even duplicative with the role of internationally-projected laboratories like the CDC, the cost effectiveness of the institutions themselves are occasionally questioned. Also, a similar question is often asked, especially by international health professional colleagues in regard to the overseas laboratories' role in influenza surveillance: why DoD? In 1996, Presidential Directive NSTC-7 directed federal agencies, including DoD, to develop a global surveillance network, enhancing research and training, engaging our international partners, and strengthening public outreach. There is an element of risk in studying and conducting surveillance for influenza (avian or otherwise). There is inherent risk (in terms of the biohazard risk of working with dangerous pathogens), and also occasionally in terms of security. It is important to have people with a Soldier's mindset, commitment, drive, and sense of duty to accomplish what must be done. Circumstances such as a pandemic may require that individuals or a force are ordered to respond in order to diagnose, attempt to contain outbreaks, or maintain order. Military transport, communications, and logistics systems may be the only such systems that remain operational, and we can rest assured that DoD public health professionals will execute such orders. With these facts in mind, the overseas laboratories have always practiced projection, engagement, and collaboration, and are experts unlike any others in the area.

With this sense of necessity, NAMRU-3 began working with host countries to enhance seasonal influenza surveillance in 1998. The following list of initiations of collaborations illustrates the exponential growth of the effort:

1998	Egypt
1999	Syria, Oman
2000	Djibouti
2001	Kazakhstan
2002	Ukraine
2003	Kyrgyzstan
2004	Saudi Arabia, Kenya, Uzbekistan
2005	Pakistan, Nigeria, Georgia, Azerbaijan
2006	Afghanistan, Bulgaria, Macedonia, Iraq
2007	Tajikistan, Turkmenistan, Ghana, Sudan, Jordan, Libya

The purpose of the effort is to identify mutated influenza viruses that have begun to circulate widely because this would compel WHO and its WHOCCs to recommend a change in the seasonal epidemic vaccine composition. Initially, we worked slowly and selectively, choosing countries with dense populations and significant human movement that we thought might be flash points for the emergence of new strains of influenza. The composition of the vaccine directly affects the health of deployed troops and Americans at home. Because only wealthy western nations had formerly conducted influenza surveillance, we had to initiate the surveillance ourselves as the only means to determine whether existing vaccines would protect troops against viruses circulating in areas in which they are deployed. However, conducting meaningful influenza surveillance is a time-consuming proposition which requires access to civilian, especially pediatric, populations in order to gain the best sense of introduction of new viruses. In other words, this is a job for on-the-ground, daily-engaged officials of national departments or ministries of health. Thus, it is only by working in true collaboration with such governmental agencies that we can achieve the goal, in other words, employing the "teach a man to fish" concept.

In general terms, the effort entails an approximate scenario of:

- Year 1: Initiation, training, capacity building
- Year 2: Technology transfer
- Year 3: Technical support

NAMRU-3's work in enhancing regional seasonal influenza surveillance has always been led by a comparative medicine specialist and has involved veterinarians in many roles. This is because NAMRU-3 recognized influenza as a zoonosis before this view was common. The increased emphasis on avian influenza required only that veterinarians already working in influenza diagnostics change focus slightly.

This slight change in emphasis has dictated that NAMRU-3 take on an increased role in capacity enhancement in the region. We recognized early on that each sovereign nation needs the tools to diagnose acute avian influenza as well as influenza in humans caused by a virus of avian origin. Serologic methods cannot meet this need; the only options are PCR or virus isolation and subtyping. Of these, only PCR provides the same day diagnosis that is essential in this area. This argument is now widely accepted by major donors, such as the World Bank, which had previously countered that donor provided PCR machines are gathering dust in many national laboratories. NAMRU-3 has always recognized that technology transfer is not a matter of simply providing equipment—it is a long-term relationship between teacher and student. Veterinarians have always served as primary trainers in NAMRU-3 capacity-building engagements.

## RESULTS

NAMRU-3 has provided avian influenza-focused, PCR diagnostics training to public health or veterinary central laboratory staff in over 40 countries, and maintains very close collaborative support for the PCR laboratories in Afghanistan, Jordan, Egypt, Libya, and Ghana. NAMRU-3 veterinarians have deployed to Bulgaria, Djibouti, Pakistan, Palestine, Sudan, Kenya, Nigeria, Ghana, Yemen, Ethiopia, Iraq (twice), Afghanistan, Ukraine, Azerbaijan, Armenia, Georgia, Turkey, and Kazakhstan to followup on suspected HPAI outbreaks. In some cases, this involves civilian veterinarians, but only uniformed staff are deployed to combat zones. Comparative medicine veterinarians have provided laboratory, biosafety, and outbreak investigation training to other veterinarians participating in these responses.

NAMRU-3 has never lost focus on the importance of surveillance for seasonal influenza. Our efforts have resulted in the characterization of thousands of isolates

which otherwise would not have reached a WHO Collaborating Center. Most importantly, isolates have been obtained from countries and regions from which no information was previously available (eg, all of central Asia). Two laboratories that were inactive prior to NAMRU-3 assistance, Ukraine and Kazakhstan, are now independently functioning WHO recognized NICs.

With the new emphasis on avian influenza, NAMRU-3 surveillance efforts also resulted in critical virus characterizations that otherwise would not have occurred. For example, viruses from Iraq, Djibouti, and Kazakhstan were fully characterized and sequenced. NAMRU-3 led the way in sharing phylogenetic information. While many institutions retained sequencing data pending completion of their own manuscripts, on the day we received host country permission, we published the sequence of the first H5N1 isolate we obtained. In August 2006, a consortium including influenza researchers at US CDC and global International Office of Epizootics\*/Food and Agriculture Organization<sup>†</sup> (OIE/FAO) reference laboratories called on others to do so.<sup>5</sup>

The first H5N1 outbreak in Afghanistan occurred in March 2006. NAMRU-3 deployed a mobile PCR laboratory which was able to diagnose the cause of the outbreak as H5 avian influenza, but government systems were not in place to respond. The government did not attempt to contain outbreaks until it received reference laboratory confirmation. Results were indeed confirmed, but the wait for confirmation caused a delay in response of over a week, during which the outbreak continued to spread. Several thousand birds in the infected villages were eventually culled, perhaps unnecessarily, given the long delay. This had a significant impact on livelihoods and confidence in the government. There is no definitive information, but the temporal and geographical spread was suggestive of introduction from Pakistan, and spread within Afghanistan through the live bird market system. Eventually the virus spread to more than 6 provinces in Afghanistan.

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\*An organization of 127 member countries headquartered in Paris, France. Information available at <http://www.oie.int>.

<sup>†</sup>An agency of the United Nations which was founded in 1946 to lead international efforts to defeat hunger. Information available at <http://www.fao.org>.

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Over the next year, NAMRU-3 worked with the Ministry of Agriculture, Irrigation, and Livestock to establish a permanent PCR laboratory in the Central Veterinary Diagnostic and Research Laboratory in Kabul. This laboratory diagnosed H5N1 reintroduction into Afghanistan in February 2007. In contrast to last year's hesitation, the government now shows total confidence in the laboratory, and immediate action was taken when positive results were obtained. Thus the novel capability of the laboratory to provide real-time results was well used. Outbreaks this year were limited to 3 provinces, and only a few hundred birds were culled. Thus, this is a success story, not merely in physically establishing laboratory capacity, but in integrating the physical capacity with government systems.

In January 2006, unusual mortality was noted in backyard chickens in Sulymaniyah, Iraq, with clinical signs consistent with avian influenza. Samples were tested with a rapid antigen detection purported to be able to specifically detect H5. The assay is a 2-step process:

1. Test for influenza A antigen and, if positive,
2. Test for H5 antigen.

The test was positive for influenza A, but not positive for H5 antigen. The villagers were advised that this was not an outbreak of H5. Approximately one week later, a young girl and her uncle, who had slaughtered sick birds, died of H5N1. As part of the followup to the outbreak investigation, NAMRU-3 examined the antigen detection assay that was used to diagnose influenza A, but did not detect H5. The influenza A antigen detection component of the assay appears to be more sensitive than the H5 component, and the H5 component does not detect all H5 viruses. Thus, there is the possibility that the assay may result in false negatives for H5 through 2 mechanisms, with the former being extremely misleading because it results in the appearance that an outbreak is due to a non-H5 avian influenza. Many non-H5 avian influenza viruses are common in the Middle East, and do not appear to be serious human pathogens.

Additional poultry samples from Sulymaniyah were submitted to NAMRU-3. The samples had been tested in Baghdad to the extent possible according to current OIE/FAO recommendations. Under the circumstances,

this was limited to serologic testing. Unfortunately, most chickens that are exposed to H5N1 die before developing antibody, so a major outbreak in a broiler barn was diagnosed as "not avian influenza" because antibody could not be detected. Samples contained high titers of H5N1 virus which are detectable by PCR.

NAMRU-3 diagnosed the cases of influenza in humans caused by H5N1 virus, and NAMRU-3 veterinarians participated in a WHO-led followup of the outbreak. The investigation resulted in diagnosis of H5N1 in poultry and cats in another Iraqi governorate, and provided opportunities to link the H5 cases in humans to disease in animals.

### **LESSONS LEARNED**

Comparative medicine veterinarians are uniquely capable of providing the broad array of expertise necessary to support all aspects of influenza surveillance, research, and response capabilities. They can address public health and infection control issues, vaccination and biosecurity policy in poultry, as well as diagnostic techniques. Veterinarians play an important role in keeping the focus of the response to the current H5N1 outbreak where it belongs—on the impact on agricultural economies, while providing a balanced approach in light of the fact that there is a potential role of this pan-zootic in precipitating a pandemic.

NAMRU-3 has made direct contributions to the body of scientific knowledge concerning avian influenza. Most virology books in print still highlight the role of migratory birds as "reservoirs" of avian influenza. NAMRU-3 work has shown that although migratory birds have probably had a role in some introductions of avian influenza into previously naïve places, poultry trade is almost certainly the predominant mechanism of transmission.

Through its training programs and collaborations, NAMRU-3 efforts bring scientists together and build bridges that may be key in responding regionally and globally to a pandemic. This effort has not only provided opportunities for exchange between host country and US scientists, but also serves to forge regional and even intercontinental relationships and understanding.

Our work builds on existing structures; we work within the WHO system internationally, and within the ministry of health or agriculture system in a given country.

## CONCLUSION

NAMRU-3 has a unique relationship with the WHO Eastern Mediterranean Region (serving as its influenza reference laboratory), and is one of the 9 global, WHO-recognized H5 reference laboratories. NAMRU-3 has unique diagnostic capacity that US Army veterinary microbiologists and other veterinarians have established, including virus isolation and subtyping in tissue culture and eggs; PCR for influenza A, B, H1, H3, H5, H7, H9, N1; full genome sequencing of influenza A viruses; neuraminidase resistance testing; and microneutralization and diagnosis of other respiratory disease (eg, severe acute respiratory syndrome).

Because the Army Veterinary Corps provides the veterinary mission for the entire DoD, US Army veterinarians already have the good fortune to work in numerous joint assignments. However, assignment to one of the overseas laboratories provides an opportunity for a vastly expanded degree of interagency experience. The comparative medicine veterinarian assigned to NAMRU-3 is expected to consult and collaborate with the US Departments of State (directly and through the US Agency for International Development and the embassies), Agriculture, and Health and Human Services (directly and through the US CDC). International agencies with which routine contact is essential include the World Health Organization, the UN Food and Agriculture Organization, and the World Bank. The most important relationships of all are with host country officials from ministries of health, agriculture, and others who have assigned roles in public health.

The next pandemic may be insidious and may be detected first through routine “seasonal influenza” surveillance. The next pandemic will probably not be

associated with disease in poultry. “Ordinary” influenza and “ordinary” surveillance systems cannot be neglected in favor of a focus on avian influenza.

NAMRU-3 is essential in implementing the National Strategy for Pandemic Influenza,<sup>6</sup> and the comparative medicine veterinarian assignment there is key in that facilitation. The strategy emphasizes the importance of capacity building, coordination, rapid response teams, and transparency. NAMRU-3 has a decade of experience implementing those themes in the area of influenza.

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# Canine Hip Dysplasia: Surgical Treatment for the Military Working Dog

CPT Kent J. Vince, VC, USA



Hip dysplasia is one of the most common orthopedic diseases in dogs. All breeds of dogs can be affected by this developmental disease. However, it mainly affects medium and large breed dogs such as the German Shepherd Dog. Pain and decreased hind limb mobility and function are the end result of this complex disease process. Severe canine hip dysplasia (CHD) can be career-ending for the Military Working Dog (MWD).

The German Shepherd Dog and Belgian Malinois make up the overwhelming majority of MWDs in America. Their high energy, trainability, intelligence, physical stature, and ideal personalities make them excellent working dogs. Unfortunately, these large breeds are often afflicted with orthopedic developmental diseases such as hip and elbow dysplasia. The Department of Defense Military Working Dog Center (DoDMWD) performs a variety of screening tests prior to the purchase of potential MWDs. Over 60% of all dogs evaluated are rejected because of behavioral or medical problems.<sup>1</sup> As one part of the screening process, radiographs of the pelvis are performed to look for signs of hip dysplasia. Dogs with signs of hip laxity or degenerative joint disease based on these radiographs or physical exam are rejected from purchase.

Several retrospective studies have evaluated the cause for retirement or euthanasia of the MWD. From 1993 to 1996, 19.5% (178/927) of all MWD removals from service were due to appendicular degenerative joint disease (DJD), primarily hip and elbow dysplasia.<sup>2</sup> From 2000 through 2004, that figure improved to 8.2% (22/268).<sup>3</sup> Several conclusions can be drawn from these figures:

1. Medical management of DJD has improved significantly to keep the MWD working longer.

2. More stringent physical exam and radiographic screening tests are performed prior to purchase, thus the DoDMWD purchases fewer dogs with developmental problems.
3. Breeders are culling undesirable developmental conditions from their breeding programs, thus producing a healthier dog for sale.

In any case, the fact that fewer MWDs are forced into retirement due to canine hip dysplasia is in the best interest of the DoD.

## DIAGNOSIS

The diagnosis of CHD is based on history, clinical signs, physical examination findings, and radiographs of the coxofemoral joint(s). Hip dysplasia often clinically presents in a biphasic process. Lameness develops initially at 3 to 10 months of age during the early phase of the disease. This pain is due to subluxation, inflammation, and synovitis induced from the hip joint laxity. Most young dogs suffering from CHD will "grow out" of their lameness close to a year of age. The pain usually returns during midlife of the dog, starting around 4 to 5 years of age. This second phase of pain is again associated with inflammation and synovitis, but in addition, the coxofemoral joint has undergone erosive cartilage changes, thickening of the joint capsule, osteophyte formation, and bony remodeling changes. The exhibited lameness can range from very mild only after strenuous activity, to very severe, such as the inability to bear significant weight or walk on the affected pelvic limb. Some owners report a "bunny hopping" run as the dog limits its coxofemoral joint range of motion to prevent exacerbation of the pain.

Physical exam findings most often elicit pain of the hip region during extension, external rotation, and



Figure 1. A ventrodorsal extended pelvic view radiograph from a 2-year-old dog without any evidence of degenerative joint disease or canine hip dysplasia.

abduction of the coxofemoral joint. Some dogs will become fearful and potentially aggressive when the examiner attempts to palpate or manipulate the hip joint. Crepitus is usually felt during the later phase of the disease as the joint has undergone significant degenerative changes. Subluxation can be felt in many young dogs. A low “clunk” is usually felt or heard during the abduction of the coxofemoral joint in young dogs as a result of the reduction of the subluxated femoral head. This clunk is referred to as an Ortolani sign and is highly suggestive for hip joint laxity. Muscle atrophy of the thigh is commonly seen in cases of CHD, but most obvious when only one leg is affected.

Several radiographic techniques have been described to screen for signs of canine hip dysplasia. The PennHIP\* distraction radiograph technique, the dorsal acetabular rim view, and the ventrodorsal pelvic view are all routinely performed. However, the most commonly performed radiograph is the ventrodorsal pelvic extended view (Figure 1, Figure 2) for diagnosis of CHD. The radiographic changes seen with canine

hip dysplasia can include subluxation of the femoral head; flattening of the femoral head; osteophytosis of the femoral head, neck, or acetabulum; sclerosis of the femoral neck; and evidence of the insertion of the joint capsule on the femoral neck.

### CONSERVATIVE TREATMENT

The goal of standard conservative management of CHD is the alleviation of hip pain. The pillars of conservative management include exercise modification, weight management and diet, pain relieving drugs, and chondroprotective agents. Adequate exercise is important for maintaining and improving muscle mass and function. This can be achieved through daily leash walk activity, moderate running, and veterinary physical rehabilitation exercises, including the use of treadmills or underwater treadmills. Disuse atrophy can often be remedied with the addition of an appropriate exercise program.

Weight management and dietary intake are two of the most important external contributing factors in CHD. It is well known that overweight or obese dogs are



Figure 2. A ventrodorsal extended pelvic view radiograph of a 6-year-old female German Shepherd Dog with severe degenerative joint disease from canine hip dysplasia.

\*University of Pennsylvania Hip Improvement Program

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often less active than normal weight dogs and the added weight puts significant strain on ligaments and joints. A lifelong study evaluated the affect of a restricted diet on the onset of radiographic evidence of hip osteoarthritis in dogs. The median age for onset of radiographic signs of osteoarthritis in dogs fed ad libitum was 6 years versus 12 years for dogs fed a 25% reduced diet. The investigators concluded that dietary restriction by 25% resulted in significant delay of the onset of radiographic signs of hip arthritis.<sup>4</sup>

Pain relieving drugs are an important weapon in the treatment of CHD. A multimodal approach to treating the pain of hip dysplasia has been used with a variety of classes of drugs. Nonsteroidal anti-inflammatories (NSAID), such as carprofen, deracoxib, meloxicam, and tepoxalin, are usually the first line of defense. They work well at reducing the inflammation within the joint which reduces the sensitivity of the nerves and results in decreased pain. Extensive research has been performed and determined that several of these NSAIDs can be safely used for long-term therapy. Tramadol is a synthetic opioid that has great benefit in relieving pain in dogs. Amantadine is an NMDA antagonist that can be used in the treatment of chronic pain. In addition, gabapentin, an antiepileptic drug, has been successfully used to treat presumed pain in dogs. A combination of an NSAID and these additional drugs can be useful in alleviating the pain induced by CHD.

Chondroprotective agents have become quite popular in the treatment of arthritic disease in dogs. These drugs aim to protect and provide the building blocks of cartilage and synovial fluid to help promote the ideal joint health. There are several glucosamine containing veterinary products available including Cosequin®, GLC 5500, and Glyco-Flex®, all aimed at promoting joint health. Adequan® is an injectable polysulfated glycosaminoglycan that helps prevent the breakdown of joint cartilage. Veterinary research showing significant improvement in dogs suffering from CHD with the administration of these chondroprotective agents is limited. Because some dogs appear to improve clinically, many clinicians advocate a trial to see if they help a specific individual.

### **SURGICAL TREATMENT**

The primary goals of surgical treatment for CHD are alleviation of hip pain and return to normal function of

the affected leg. Surgery is often used in conjunction with medical management. Total hip replacement (THR) and femoral head ostectomy are the 2 primary surgical options for treatment of hip dysplasia. Surgery is usually performed when medical and conservative management of the disease is no longer successful.

Femoral head ostectomy is typically thought of as a last resort salvage procedure in dogs suffering from severe hip dysplasia. In this surgery, the femoral head and portions of the neck are cut and removed thus eliminating the bone on bone contact of the coxofemoral joint. The muscles and soft tissues surrounding the proximal femur and acetabulum will form a “false” joint. The long-term return to function of the affected leg is variable and often dependent on several factors including the size and body condition of the dog. While dogs undergoing femoral head ostectomy will not have 100% normal function of the affected leg, nearly 90% of owners reported a good outcome with this procedure.<sup>5</sup>

Canine total hip replacement is the best surgical option for returning an affected coxofemoral joint to normal function. THR has been performed in both a research and commercial setting in canine patients for several decades. Initial metal implants were fixed with bone cement into the femur and acetabulum. This technology has gone under several improvements and advancements over the past 30 years and is still used today in both dogs and humans. Newer technology has led to the development of uncemented implants. Porous-coated implants are press fitted into the proximal femur and acetabulum. The bone grows into the porous coating, permanently stabilizing the implant in approximately 2 to 4 weeks. A long-term study evaluating the use of uncemented porous-coated THR implants in dogs revealed an 87% success rate. The authors concluded that after a 6-year follow up, uncemented fixation of femoral stem and acetabular cup implants was successful.<sup>6</sup>

At the North Carolina State University Veterinary Teaching Hospital, the implant of choice is the BFX™ (biological fixation) total hip replacement system by BioMedtrix (Boonton, New Jersey). This system uses a cobalt chrome femoral stem with 3 layers of micro beads surrounding the proximal third of the implant (Figure 3). The femoral head component is a highly polished cobalt chrome sphere that is lightly

hammered onto the femoral stem component. The acetabular cup component has an outer shell made of titanium with 3 layers of micro beads and an ultrahigh molecular weight polyethylene liner (Figure 4). The layers of micro beads create a porous coating on the implants that allow for bony ingrowth and when healed, a very stable implant-bone interface. In human total hip replacements, the implants are expected to last greater than 20 years. Thus, the BFX total hip replacement implants are expected to last the lifetime of the dog.

Proper patient selection and surgical planning are imperative for surgical success. The patient must be free of any systemic diseases or infections that could potentially spread to the implants, as implant associated infection would result in failure. The patient must be well-trained and sensible so as to tolerate small cage/kennel confinement and controlled activity during recovery. Any excessive activity too early in the postoperative recovery phase could lead to implant movement, implant associated bone fracture or luxation. While there are a variety of implant sizes to accommodate most medium and large breed dogs, the patient must be of appropriate size to ensure proper implant fit. Preoperative radiographs are used to estimate the size of femoral and acetabular implant and to give the surgeon an idea of how much bone to remove during the preparation of the bone bed. The patient should be free of any neurological conditions that might affect the use of the hind limbs such as lumbosacral disease. The patient should also be free of any other orthopedic disease affecting either one of the pelvic limbs, such as cranial cruciate ligament rupture.

The surgical procedure for canine total hip replacement is technically challenging and should only be performed by a highly qualified veterinary surgeon. During the



Figure 3. The BFX femoral stem implant

or, even worse, implant associated bone fracture or luxation. While there are a variety of implant sizes to accommodate most medium and large breed dogs, the patient must be of appropriate size to ensure proper implant fit. Preoperative radiographs are used to estimate the size of femoral and acetabular implant and to give the surgeon an idea of how much bone to remove during the preparation of the bone bed. The patient should be free of any neurological conditions that might affect the use of the hind limbs such as lumbosacral disease.



Figure 4. The BFX acetabular cup implant

procedure, the anesthetized patient is placed in a pelvic positioning device to aid in appropriate implant alignment. Strict aseptic technique, perioperative antibiotics, and surgeon sterility are vital in preventing surgical associated infection. A craniolateral surgical approach is made to the coxofemoral joint. The head of the femur is precisely cut to expose the femoral canal and also to provide increased exposure to the acetabulum. The acetabulum is sequentially reamed and shaped to accurately prepare the bone bed for the implant. Once the bone bed is prepared to the proper size, the porous-coated titanium acetabular implant is seated and hammered into position and correct orientation. The femoral canal is then drilled and shaped with a series of graduated broaches to prepare the femoral bone bed. Once the appropriate canal preparation is attained, the femoral stem is impacted into the proximal femoral canal. A trial size femoral head is then placed on the femoral stem to determine the appropriate femoral head implant. Different femoral heads allow the surgeon to lengthen or shorten the femoral neck, thus helping to minimize the possibility of postoperative coxofemoral luxation. Once the appropriately sized head has been placed, the femur is reduced and the joint capsule is closed. The muscles, soft tissues, and skin are closed in a routine manner and an adhesive bandage is applied over the surgical incision. Postoperative radiographs are made to ensure appropriate orientation and alignment of the implants.

The postoperative recovery period is imperative to surgical success of the total hip replacement procedure. All dogs are administered at least 2 types of drugs to aid in pain relief during the first 2 to 4 weeks of recovery. Most dogs are toe-touching lame the day after surgery. The patient will often bear significant weight on the affected

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limb the second day after surgery. Owners are instructed to use a sling placed under the dog's abdomen to help prevent a fall while the dog is walking and to help control the patient if they try to be too active. The dog is strictly confined and only allowed to go outside on leash for bathroom use for the first 4 weeks. During the second month, short 5-minute leash walks are performed twice daily and gradually increased to 30 minutes by the end of the month. During the third month, the leash walk activity is continued and a small amount of supervised off-leash running is permitted. Postoperative recheck examinations are performed at 3, 6, and 12 months. Radiographs are made to evaluate the implants for any signs of movement, bone reaction, or possible infection. If no problems are detected on physical exam or radiographs at 3 months, the dog is permitted to return to normal activity and training.

While the complication rate with the BFX total hip replacement system is low, the complications can be significant. Postoperative coxofemoral luxation, femur or pelvic fracture, implant subsidence or movement, and implant infection are all possible THR complications. Coxofemoral luxation is usually treated with reoperation and the placement of an iliofemoral suture to help prevent craniodorsal luxation. Femur or pelvic fractures are usually treated with reoperation and internal fixation with a bone plate and screws. Depending on the degree and severity of the implant movement or subsidence, surgery may not be indicated. In extreme cases of implant movement, reoperation is usually performed, and either a larger implant is placed or a cemented implant is used. In the very rare case that an implant associated infection develops, bacterial culture is performed and antibiotic therapy instituted. If the infection fails to resolve, the implants usually must be surgically removed.

### **CASE REPORT: MWD BENNY**

At the time of original presentation to the North Carolina State University Veterinary Teaching Hospital (NCSU VTH), MWD Benny was a 5-year-old, male, German Shepherd Dog certified in patrol and explosives detection stationed at Marine Corps Air Station, Beaufort. He had a 3-month history of right hind limb lameness during training and working. He had been previously treated by the attending Veterinary Corps Officer at the Marine Corps Recruit

Depot, Parris Island, with carprofen (Rimadyl<sup>®</sup>) 100mg orally every 12 hours, Cosequin 2 tablets twice daily, and exercise restriction. His physical exam findings at the NCSU VTH included mild lameness of the right hind leg while walking, pain on extension of the right coxofemoral joint, slight pain on extension of the left coxofemoral joint, positive Ortolani sign of the right coxofemoral joint, and mild right hind leg muscle atrophy when compared to the left hind leg. Complete blood count and blood serum chemistry indicated elevated cholesterol (454mg/dL ref. range 92-324) with all other values within normal limits. Radiographs performed at NCSU VTH revealed pronounced left coxofemoral DJD with osteochondral fragments, bilateral mild coxofemoral subluxation, and mild right femoral head remodeling (Figure 5). On the basis of physical exam and radiographic findings, the diagnosis of coxofemoral DJD and hip dysplasia was made. Since MWD Benny's lameness and pain response were more severe on the right hind leg, a total hip



Figure 5. Preoperative ventrodorsal extended pelvic view of MWD Benny. Despite the moderate degenerative joint disease changes in head of the left femur, there is radiographic evidence of disuse muscle mass atrophy in the right leg compared to the left.

replacement was performed only on the right coxofemoral joint.

A BFX modular total hip replacement system was used in MWD Benny. His ideal temperament, outstanding drive, and excellent detection abilities made him the ideal patient. The anesthetized patient was placed in a pelvic positioning device on the surgery table to ensure appropriate alignment. A modified craniolateral approach to the right coxofemoral joint was made. The femur was externally rotated, the round ligament was cut, and the femur luxated to expose the femoral head. The neck cutting guide was positioned and a femoral neck cut was made at the level of the lesser trochanter with an oscillating bone saw. The femoral head was removed. With the acetabular cup exposed, a set of sequentially sized reaming devices were used until the medial acetabular cortex was identified. A 26mm BFX acetabular cup was placed and seated within the right

acetabulum. The femur was then elevated and the caudal and lateral femoral neck was removed with rongeurs. The femoral canal was opened with a 5mm drill bit and then sequentially enlarged with #6, #7, #8, and #9 broaches. A #9 BFX femoral stem was impacted and firmly seated in the right femur. A +3 femoral head was lightly hammered onto the femoral neck. The joint was reduced and the limb could externally rotate 90° without luxation. The joint capsule, muscles, subcutaneous tissues, and skin were closed in a routine manner. Postoperative radiographs were taken to assess implant placement and positioning. The patient recovered from anesthesia without complications. Hydromorphone and medetomidine were administered postoperative for 24 hours as needed for pain relief.

MWD Benny was discharged approximately 48 hours after surgery. Carprofen 75mg orally every 12 hours and Cosequin 2 tablets twice daily were prescribed. Strict kennel confinement and activity restriction was mandated for 4 weeks. During the second month of recovery, MWD Benny's activity included leash walks 2 to 3 times a day, starting at 5 minutes and increasing progressively to 30 minutes by the end of the month. During the third month, activity continued to increase to allow for short periods of off-leash running on a daily basis and continually working up to resume a near normal presurgery level of exercise.

At the recheck examination 3 months postoperative, MWD Benny was using the right leg with no observed lameness in either limb. There was no pain on palpation or manipulation of the right pelvic limb with range of motion within normal limits. There was mild muscle atrophy of the right hind leg compared to the left. Radiographs did not detect any significant change in the implants or the bone from the radiographs taken immediately after surgery. MWD Benny was authorized to return to full activity.

At the 6-month recheck examination, MWD Benny had returned to normal training (obstacle course) and activity (patrol/attack work) without any lameness or problems noted by the handler. There was no lameness or pain detected in the pelvic limbs on physical exam. Current radiographs did not detect any significant change from the radiographs taken immediately after surgery.



Figure 6. Ventrodorsal extended pelvic view of MWD Benny at 17 months postoperative

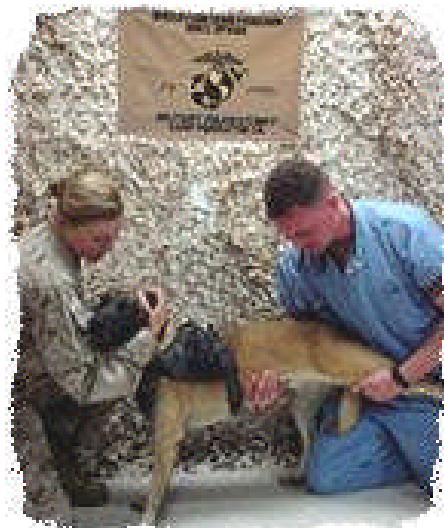
## **Canine Hip Dysplasia: Surgical Treatment for the Military Working Dog**

MWD Benny returned to the NCSU VTH 17 months postoperative for a recheck examination. He had just returned from an 8-month deployment to Iraq and performed without complications during his tour of duty. Physical exam again did not detect any abnormalities, lameness, or pain in either pelvic limb. Radiographs did not detect any changes from the radiographs made 11 months earlier (Figure 6). DJD of the left coxofemoral joint was still present but unchanged from earlier radiographs.

MWD Benny made a total of 3 deployments to Iraq after his total hip replacement in February 2004. He has not developed any clinical lameness affecting his right hind leg associated with the total hip replacement. Unfortunately, MWD Benny developed significant lameness of his left pelvic limb due to the progression of degenerative joint disease and hip dysplasia during his third deployment and returned home early.

### **CONCLUSIONS**

Total hip replacement for the treatment of canine hip dysplasia is highly successful at alleviating pain and returning the affected limb to normal function. Canine hip dysplasia has been the most common medical cause of early retirement for MWDs. The BFX uncemented total hip replacement provides a pain free normal functioning coxofemoral joint in the dog. In special cases of well-mannered, highly skilled, and technically proficient MWDs afflicted with severe canine hip dysplasia, total hip replacement surgery can be successfully performed to significantly extend the pain-free working career of the dog.



### **ACKNOWLEDGEMENTS**

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# A Clinical Trial of Ivermectin Against Eyeworms in German Shepherd Military Working Dogs

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## ABSTRACT

**Objective:** To determine if monthly ivermectin was efficacious in reducing the observed incidence of eyeworms over a period of 2 months as compared with normal husbandry practices in a population of Republic of Korea Army military working dogs (MWDs).

**Methods:** Prospective observation of 114 German Shepherd MWDs in a randomized, double-blind, controlled clinical trial. MWDs were randomly assigned to either a treatment group receiving a monthly dose of 0.2 mg/kg BW ivermectin orally, or to a control group given an equivalent dose volume and frequency of a saline placebo. A quantitative numerical count of eyeworms found in the eyes of MWDs was conducted at 25-day intervals.

**Results:** The prevalence of eyeworms in the treatment group went to zero at 25 days and remained lower at 50 days (5%) than baseline (24%). Prevalence in the controls remained approximately constant over all treatment times (14% to 18%).

**Conclusion:** Although ivermectin does not prevent dogs from being infected with eyeworms, the study suggests that ivermectin administered orally at a dose of 0.2 mg/kg every 3 weeks significantly reduces the prevalence of *Thelazia* species eyeworms in dogs.

## INTRODUCTION

Military working dogs (MWDs) have long been used as an effective and reliable force multiplier for military ground forces. As with any other combat system, maintenance is essential to assuring sustained peak effectiveness. In the case of MWDs, this maintenance is their medical care and management. Surveys made of the most common medical problems encountered in Republic of Korea (ROK) Army MWDs reveal several conditions that potentially can be efficiently medically managed (Table 1). Among these medical problems is an eyeworm infestation, *Thelazia callipaeda*. Medical reviews of the ROK Army MWD population suggest a 90% incidence of eyeworms. These dogs also maintain a high incidence of tick infestation.

*Thelazia* species eyeworms are a species of small round worms principally found in or around the eyes of several animals.<sup>1(p80)</sup> The worm's cuticle shows well-marked, coarse transverse striations, which look

tooth-like in profile.<sup>2</sup> *Thelazia callipaeda* occurs in Asia in the membrana nictitans of the dogs, and less frequently in rabbits and man.<sup>3-6</sup> The life cycle of most *Thelazia* species depends upon flies as intermediate hosts and vectors. The intermediate hosts and vectors for *Thelazia callipaeda* are generally unknown<sup>3</sup>; however, the houseflies of genus *Muscus* or *Fannia* probably serve as the intermediate host.<sup>7</sup> Eyeworm

Table 1. Most Common Medical Problems Found in Korean Army Military Working Dogs

Trauma
Gastroenteritis with diarrhea
ENT (Conjunctivitis)
Fungal infection/bacterial infection
Hip dysplasia (usually under 6 years of age)
Heartworms (20%)
Eyeworms (90%)
Ticks

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presence in the conjunctival sac can result in clinically relevant photophobia, blepharospasm, excessive lacrimation or a mucopurulent ocular discharge, keratitis, or corneal ulcer.<sup>7</sup> Mechanical damage to the infected dog's eyes is likely caused from the worm's coarse cuticle as it moves about the ocular tissues. If not removed, eyeworms are reported to cause blindness, presumably through production of corneal opacity. The diagnosis is made by finding and identifying the parasites in the conjunctiva.<sup>3</sup> Treatment involves manual removal of the worms with forceps or flushing after application of topical anesthesia. Aftercare consists of symptomatic treatment of the conjunctivitis and keratitis, if present.<sup>1(p80),7</sup>

Ivermectin is an avermectin class anthelmintic commonly used for roundworms (nematodes) and arthropods. Its mode of action involves the neurotransmitter gamma-aminobutyric acid (GABA). In roundworms, ivermectin stimulates the release of GABA from nerve endings and enhances binding of GABA to special receptors at nerve junctions, thus interrupting nerve impulses. This action paralyzes and kills the parasite. The enhancement of the GABA effect in arthropods such as mites and lice resembles that in roundworms except that nerve impulses are interrupted at the neuromuscular junction. This also leads to paralysis and death of the parasite.<sup>8,1(p263)</sup>

The principal peripheral neurotransmitter in mammals, acetylcholine, is unaffected by ivermectin. Ivermectin does not readily penetrate the central nervous system of most mammals where GABA functions as a neurotransmitter. Recommended doses of ivermectin have a wide safety margin in most mammals, including dogs. In certain dogs, particularly Collie breeds, the concentration of ivermectin in the central nervous system following treatment is greater than it is in other dogs. This is possibly due to a more readily penetrated blood-brain barrier in Collies, or to sequestration. In these dogs, the apparent potentiation of inhibitory neurotransmitter, GABA, by ivermectin has resulted in adverse reactions: mydriasis, depression, ataxia, drooling, paresis, recumbency, excitability, stupor, coma, and death.<sup>8</sup>

Ivermectin was shown to significantly reduce the geometric mean in the number of eye worms (*Thelazia* species) collected from the surface of eyes in treated cattle.<sup>9</sup> Kennedy<sup>10</sup> and Soll et al<sup>11</sup> demonstrated >99% efficacy of the injectable formulation of ivermectin

against *Thelazia skrjabini* in cattle. Kennedy<sup>12</sup> later demonstrated >99% efficacy of topical ivermectin against the same species eyeworm in cattle. We theorized that ivermectin would be similarly effective in working dogs.

The primary objective of this study was to determine if monthly ivermectin was efficacious in reducing the observed incidence of eyeworms over a period of 2 months as compared with normal husbandry practices without the use of ivermectin in a population of ROK Army MWDs. Secondary objectives of this study were to ascertain, in this same population of MWDs, if the same monthly administration of ivermectin would reduce the number of ticks found on the MWDs. Lastly we hoped to estimate the heartworm prevalence in ROK Army MWDs.

## MATERIALS AND METHODS

**Study Population and Setting.** The study population consisted of MWDs stationed in the First Republic of Korea Army (FROKA) near Chunchon, ROK. All studied FROKA MWDs were German Shepherds. This study was performed in the medical facilities of the FROKA Military Working Dog Training Center. Approval for this study was obtained from ad hoc medical review committees from both the US Army 18th Medical Command and the FROKA General Staff.

**Inclusion and Exclusion Criteria.** All MWDs which were assigned to the FROKA and were greater than 6 months of age were considered for enrollment as subjects in this study. Dogs were excluded if they were unable to complete the study.

**Group Assignments/Treatments.** Dogs were assigned via simple computer randomization into either a treatment or control group. Treatment was defined as the oral administration of 0.2 mg of ivermectin per kg body weight. The control group was given an equivalent dose volume and frequency of a saline placebo. All monthly doses were administered by trained US Army Animal Care Specialists.

**Data Collection and Quality.** We prospectively observed 114 MWDs in a randomized, double-blind, controlled clinical trial. Baseline data consisting of sex, age, weight, and general medical condition of the

dogs was collected at the start of the study. Each MWD selected was examined for the presence of eyeworms. If observed, the worms were mechanically removed and the eye was lavaged with saline. A quantitative numerical count of eyeworms found in the eyes of MWDs was conducted at 25-day intervals, days 0, 25, and 50. A similar count was taken of ticks found on the body of the MWDs at the same interval. Ticks were left on the dogs. Blood was drawn upon entry into the study and examined for the presence of heartworm antigen and larvae. After all numerical counts were taken, drug (saline or ivermectin) was administered and dogs were observed for any potential adverse reactions. All medical interventions and numerical counts of parasites were conducted by trained US Army animal care specialists. Handling and restraint of MWDs were conducted in the usual manner by trained ROK Army Soldiers. Neither the technicians nor the handlers were aware of group assignment or medication administered to the dogs. Dogs were positively reassured throughout the intervention period. Administrative recording of data and blinding were conducted by separate US Army administrative and logistic specialists. On-site oversight of the entire study was performed by Veterinary Corps officers from both the US and ROK Armies.

**Baseline Comparisons.** Baseline data included: age, sex, body weight, presence and number of eyeworms, presence and number of ticks, presence of heartworm serum antigen (SNAP Canine Heartworm PF Antigen Test\*), and presence of heartworm microfilaria (Difil test†).

#### Response Variables

- **Eyeworms**—The response variable was continuous, a quantitative numerical count of eyeworms found in the eyes of MWDs at 25 days intervals.
- **Ticks**—The response variable was continuous, a quantitative numerical count of ticks found on MWDs at the same 25 day intervals.
- **Heartworm**—The response variable was qualitative, a dichotomous (positive or negative) presence of serum heartworm antigen as

determined by assay or microfilaria as determined by Difil test.

**Data Analysis.** Baseline data was analyzed using a Z-test for difference of proportions and a Student's T-test for difference of means. Eyeworms were analyzed using graphic proportions (proportion infected). Drug efficacy was based on a geometric mean, and eyeworm burden was assessed using a Wilcoxon nonparametric test. Cure rate was determined by the proportion infected. Heartworms and ticks were analyzed using a Z-test for difference of proportions. Significance was set at  $P<0.05$ .

#### RESULTS

One hundred twenty-one MWDs were enrolled into the study with 114 being analyzed. Of those, 3 MWDs died of unrelated disease, 2 dogs became ill from unrelated illness and were excluded, one dog departed the study early to return to duty, and one dog was removed from the study due to disposition. With the exception of males to females, the 2 groups were absolutely comparable. Male dogs made up 63% of the treatment group (SEM<sup>‡</sup> 6.4%) as compared to 46% male population of the control group (SEM 6.7%). The proportion of males to females was not significantly different at  $\alpha=0.05$ , but was close with a  $p=0.067$ . There were no suspected biological implications to canine hosts with regard to eyeworms, ticks, or heartworms and the sex of the host. Treatment dogs had a mean age of 30.5 months (SEM 3.0). Control group mean age was 31.7 months (SEM 3.4). Mean weights for the treatment group were 28.4 kg for the treatment group (SEM 0.7) and 28.3 kg for controls (SEM 0.7).

**Eyeworms.** Prevalence of eyeworms in MWDs of each group is shown in the Figure. No difference in numbers of eyeworms between groups was observed at day 0 ( $p=0.16$ ). Prevalence in the controls remained approximately constant over all treatment times (14% to 18%) with no significant difference observed in overall baseline proportions. The prevalence of eyeworms in the treatment group went to zero at 25 days and remained lower at 50 days (5%) than the 24% seen at baseline. Combined treatment proportions were significantly less than both combined baseline and control proportions ( $p<0.005$ ).

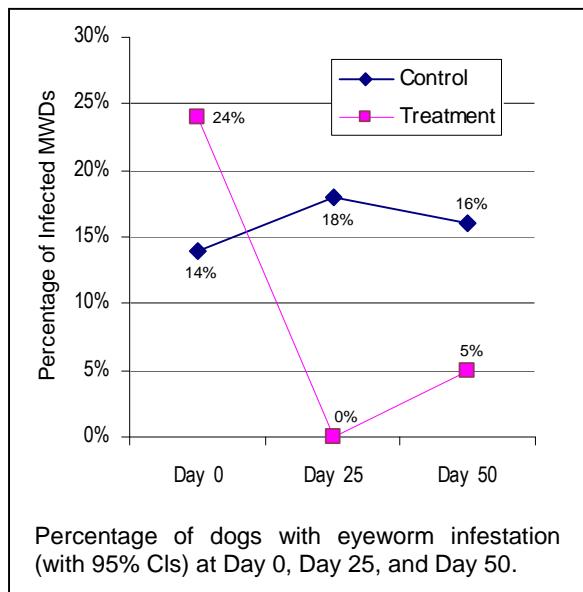
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\*IDEXX Labs, One IDEXX Drive, Westbrook, Maine 04092

†EVSCO Pharmaceuticals, Buena, New Jersey 08310

‡Standard Error of the Mean

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Due to the lack of independence between groups, the number (or proportion) of dogs who, over a given time interval, recover (eg, they had greater than one eyeworm at time X, but had zero eyeworms at time X+1), and develop the infection (eg, they did not have eyeworms at time X, but had greater than one eyeworm at time X+1) were observed. These observations, depicted in Table 2, present the number of animals that would normally recover over an interval, and the number of animals expected to become infected over an interval. In the treatment group, 4 new cases of eyeworms were observed between day 25 and day 50. Only one of these four was reinfected (ie, initially had eyeworms at the start of the study, then had no eyeworms during the first interval, and subsequently did have eyeworms again during the last interval). Of the control dogs, there were 7 dogs that developed eyeworms between day 25 and day 50. Three of these seven were reinfections.

Ivermectin efficacy was 100% at Day 25 and 71.5% at Day 50. Cure rate at Day 25 showed 100% recovery with ivermectin in the treatment group, with 14 MWDs initially infected with eyeworms and 14 improved. Control dogs showed a 25% recovery with the placebo over the same period,

Table 3. Mean Number of Eyeworms Per Dog With Eye Infestation\*

Control Group			
	Baseline	Day 25	Day 50
<b>Number</b>	9	11	8
<b>Mean</b>	16.6 (8.7)	3.2 (0.8)	3.4 (0.9)
Treatment Group			
	Baseline	Day 25	Day 50
<b>Number</b>	13	NA	4
<b>Mean</b>	18.8 (8.4)	NA	3.3 (1.7)

\*At least one eyeworm in the dog

with 8 MWDs initially infected with eyeworms and 2 improved.

The mean number of eyeworms per dog, given that the dog had one or more eyeworms, did not appear different between groups, with the exception of the treatment group at Day 25 as no infected dogs were observed (Table 3).

**Ticks.** There was no significant difference between groups regarding ticks. Prevalence and number of ticks per dog increased over time in both groups. No association was found between the number of ticks or presence of ticks and eyeworms.

**Heartworms.** Heartworm seroprevalence was 16% in treatment dogs and 18% in controls. Only one control dog was positive for heartworm microfilaria at the beginning of the study. No association was found between the presence of heartworms or presence of heartworms and eyeworms or treatment groups. Animals with heartworms were not more likely to have eyeworms, whether receiving ivermectin or not.

## DISCUSSION

The results of this study suggest that ivermectin administered orally at 0.2 mg/kg every 3 weeks appears to be effective in reducing the prevalence of *Thelazia callipaeda* in dogs. These findings are consistent with similar studies

Table 2. Proportion of New Cases\* and Recoveries† per Study Interval

Interval 1 (Day 0 to Day 25)		
	New Cases	Recoveries
<b>Control</b>	7.1 (3.4)	5.4 (3.0)
<b>Treatment</b>	0 (NA)	22.4 (3.4)‡
Interval 2 (Day 25 to Day 50)		
	New Cases	Recoveries
<b>Control</b>	8.9 (3.8)	14.3 (4.7)
<b>Treatment</b>	6.9 (3.3)	NA§

\*New cases were defined as zero eyeworms at time X, but at least one eyeworm at time X+1.

†Recovery was defined as the presence of at least one eyeworm at time X, but zero eyeworms at time X+1.

‡ All dogs that initially had eyeworms recovered.

§ Since no treatment dogs were infected at Day 25, none could have recovered in the succeeding interval.

in cattle involving *Thelazia* species where the efficacy of ivermectin in the medical treatment of the eyeworms was demonstrated. These studies showed a 97% to 100% efficacy in cattle at 8 to 14 days post treatment with ivermectin. Immature worms typically returned at 22 days post-treatment. Multiple routes of administration were used (subcutaneous injection and topical pour-on) over a dosage range from 0.2 to 0.5 mg/kg body. Soll et al noted that although ivermectin administered subcutaneously was >99% effective against *Thelazia rhodesii*, 7 of 16 cattle they examined became reinfected 22 days following treatment.<sup>11</sup>

The high efficacy of the injectable formulation of ivermectin also demonstrated in other studies in cattle<sup>8,1(p263)</sup> suggests infection was reestablished by immigrating infected flies. Face flies can migrate 1-2 kilometers within 24 hours, implying that an effective fly abatement program would be a necessary component of an overall eyeworm control program for working dogs housed and worked outdoors.

An added benefit of using ivermectin as a part of an eyeworm prevention program is that it would be coincidentally effective as a heartworm preventive. The dosage of ivermectin reported here is approximately 10 times the usual dosage used for heartworm prevention. The observed dogs had a notable sero-prevalence suggesting a heartworm prevention program would be beneficial.

Ivermectin, and the avermectins as a group, are relatively inexpensive, widely available, and easy to use veterinary drugs. Effective over a wide dosage range and via multiple routes of administration, ivermectin provides multiple therapeutic modality options for the care provider. Although a relatively older drug, and somewhat limited in use in some canine breeds, ivermectin offers a good management choice/alternative for German Shepard working dogs. Further study should explore the potential use and efficacy of newer, safer, and even easier to use avermectin class drugs.

It should be noted that, although not common, *Thelazia* infestations are possible in humans. This makes eyeworm control a potential consideration for those working in areas of increased likelihood of exposure.

## LIMITATIONS

This study was significantly limited in regard to its observations of ticks. Better observations as supported by similar studies in other animal species would have been in noting the genus/species of ticks found on the MWDs, volume of blood consumed by the ticks, average body weight of ticks after ivermectin treatment, and perhaps tick egg production after ivermectin. Although ivermectin use in animals has been shown to reduce the abundance of all stages of ticks in pastures,<sup>13</sup> reduce the body weight and amount of blood consumed by ticks,<sup>14</sup> and reduce subsequent egg production of ticks,<sup>15</sup> reduction in tick numbers on treated animals were not readily apparent.<sup>16</sup>

## CONCLUSION

Although ivermectin does not prevent dogs from being infected with eyeworms, this study suggests that ivermectin administered orally at a dose of 0.2 mg/kg every 3 weeks reduces the prevalence of *Thelazia* species eyeworms in dogs. Used in concert with a practical fly abatement program in endemic areas, ivermectin could effectively manage eyeworms in working dog populations.

## ACKNOWLEDGEMENT

The successful outcome of this study is in large part due the diligent efforts of the Soldiers on the FROKA Military Working Dog Center and the 129th Medical Detachment (Veterinary Medicine). Of particular note is the work of MSG (Ret) Frank Rinker in coordinating the logistics and movement of personnel, and SGT Kim Yong in providing interagency translation and coordination support.

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# Using Predictive Microbiology to Evaluate Risk and Reduce Economic Losses Associated with Raw Meats and Poultry Exposed to Temperature Abuse

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## ABSTRACT

The Department of Defense suffers economic losses when temperature-abused raw meat and poultry are condemned. Current US Army guidance regarding time/temperature limits associated with these foods (RISK-3 category) is ultraconservative, especially at lower temperatures. We have developed a more accurate, yet conservative or “fail-safe” computer-based tool for predicting pathogen growth in raw meat and poultry. In 20 trials of this tool, growth ( $>0.3$  log colony-forming unit increase) or no growth of *Salmonella* serovars, *Escherichia coli* O157:H7, and *Staphylococcus aureus* was accurately predicted 67% to 95% of the time for inoculated and temperature-abused poultry products and ground beef. Fail-safe predictions were obtained in trials for which the tool was inaccurate. The predictive tool is ready for further validation trials and field testing. Using this tool as a supplement to the current guidance will decrease losses associated with the condemnation of raw meat and poultry products exposed to short-term temperature abuse.

## INTRODUCTION

The US Army Veterinary Service, the Department of Defense Executive Agent for Food Safety, reports tens of thousands of dollars in condemned raw meat (beef and pork) and poultry losses each year. This estimate is likely considerably lower than the actual losses. Most often condemnation results from refrigeration failure or poor transportation and warehousing/storage practices which allow the product temperature to rise above the 5°C (41°F) cold-holding requirement. The primary reference for this cold-holding requirement is the Food and Drug Administration’s Food Code,<sup>1</sup> which is cited in the food safety regulations of all branches of the US armed forces. Another criterion often tied to this cold-holding requirement is that exposure of potentially hazardous foods (raw meat and poultry) to an out-of-temperature condition should not exceed 4 hours, though this is not specifically detailed in the section of the Food Code covering cold-holding of potentially hazardous food. The 4-hour criterion likely comes from another section of the Food Code addressing the use of time only as a public health

control rather than time in conjunction with temperature. However, this section applies specifically to ready-to-eat foods or to a working supply of raw foods just before cooking, both intended for immediate consumption. Thus the criterion does not apply to situations such as refrigeration failures or improper transportation/storage practices in which raw meat and poultry have been exposed to temperatures above 5°C (41°F) for any period of time. Since the Food Code is written in a manner that establishes inflexible limits for regulatory control, it does not offer any deviation guidance which would be required to make appropriate disposition decisions in these out-of-temperature situations. The current decision making tool for this purpose, historically referred to as the “Natick Refrigeration Failures Guide” (The Guide), has been incorporated into chapter 5 of the US Army Medical Command’s *Pamphlet 40-13*.<sup>2</sup> It categorizes temperature-abused foods as either SAFE or RISK. SAFE foods are those items for which refrigeration is used as a quality control measure and not for the control of pathogen growth. Examples include fresh fruits and vegetables, frozen bakery items, processed

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and hard cheeses, etc. RISK category foods (refrigeration controls pathogen growth) are broken down into 3 groups and a flow chart to determine an item's RISK group is in The Guide. Raw meat and poultry would be classified as subgroups of RISK-3 foods. Time/temperature limits for the 3 RISK groups are also in The Guide and provide a maximum exposure time (in hours) for each RISK group given in 1°C intervals for a 6°C to 25°C (42°F to 77°F) range. In summary, the time/temperature guidelines for RISK-3 foods (meat and poultry) are exposure for not more than 4 hours at temperatures of 6°C to 22°C (42°F to 72°F) or not more than 3 hours at temperatures of 23°C to 25°C (72°F to 77°F). Our research suggests that these guidelines are ultraconservative for raw meat and poultry products, especially at the lower temperature range. Having a tool to accurately predict pathogen behavior in raw meat and poultry could drastically reduce losses associated with condemnation of these temperature-abused foods. We developed such a tool, THERM (Temperature History Evaluation of Raw Meats), to address the need for small and very small meat and poultry processors to validate their Hazard Analysis and Critical Control Point\* critical limits and to provide them with a decision-making tool for process deviations.

### **TOOL DEVELOPMENT**

The development of THERM was described in Ingham, et al.<sup>3</sup> It is based on isothermal inoculation experiments in raw meat. The meats used in the isothermal studies were raw whole muscle pork, beef, and turkey obtained from a local retail store or directly from a local wholesale distributor. The meat was trimmed of fat and ground in our laboratory. Levels of indigenous microflora, fat, protein, water, and salt were determined. Then the ground meat was portioned and frozen at -20°C until used in isothermal studies. Inocula were prepared by combining each of 5 cultured (stationary phase) strains of *Escherichia coli* O157:H7, *Salmonella* serovars and *Staphylococcus aureus*. For the pork and beef isothermal studies *E. coli* O157:H7 and *Salmonella* serovars were combined into one inoculum and *S. aureus* was a separate inoculum. For isothermal studies using turkey, separate inocula of *Salmonella* serovars and *S. aureus*

were used; *E. coli* O157:H7 was not used. Isothermal studies were conducted at approximately 2.8°C (5°F) intervals over a 10°C to 43.3°C (50°F to 110°F) range. Ground raw pork, beef, and turkey were weighed (about 25 g) into small sample bags and allowed to reach the test temperature either in a static water-bath (temperatures above room temperature) or an incubator (temperatures at, or below, room temperature). When the test temperature was reached, each meat sample was inoculated with 100 µL of the appropriate inoculum. Each inoculated sample bag was sealed and manually massaged for 20 seconds to distribute the inoculum throughout the meat mass. Bags of inoculated product were returned to the isothermal experiment temperature as quickly as possible (<5 minutes). Three concurrent trials were conducted at each test temperature for all meat/pathogen combinations. Three bags per inoculum type (one per trial) were removed at each sampling time from the water bath or incubator. The outer surface of each bag was sanitized with 70% ethanol and allowed to dry. Once dry, the contents of each sample bag were transferred to a separate, large, filtered bag. The original sample bag was everted to expose any inoculum still on the bag, and was then placed into the filtered bag. The ground meat sample and original sample bag were diluted, stomached at normal speed for 30 seconds, and the resulting meat homogenate was serially diluted. Similar sampling and initial sample homogenization was done at each sampling time in experiments to test THERM, as detailed later in this article. For each dilution, 100 µL of appropriately diluted sample was spread on a single plate. The selective medium used for *E. coli* O157:H7 was Sorbitol MacConkey agar on which typical colonies are colorless-to-white and opaque. The selective medium used for *Salmonella* serovars was Xylose Lysine Deoxycholate agar on which typical colonies have a black center and a well defined clear-to-opaque halo. The selective medium used for *S. aureus* was Baird-Parker agar base with tellurite egg yolk supplement on which typical colonies are shiny black with a distinctive clear zone in the surrounding agar. For each meat/pathogen combination and test temperature, the log colony-forming unit (CFU)/sample was determined at each sampling time for each of the 3 trials. The sampling time and log CFU/sample data were then entered into the DMFit<sup>®</sup> 2.0 program (J. Baranyi,<sup>4</sup> Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, UK) which generated a best-fit growth curve, with an estimated

\*A program of the US Food and Drug Administration to institute a uniform standard for food safety tests and inspections. Information available at <http://www.cfsan.fda.gov/~comm/haccpov.html>.

lag phase duration (LPD), growth rate (GR), and corresponding R-squared value. The LPD and GR values for each meat/pathogen combination were used to develop the THERM tool. A software application was written which allows the user to enter up to 20 elapsed-time (minutes) and temperature (°F) data pairs. The application uses an interval accumulation strategy to estimate the percent of LPD elapsing in each time interval (constant temperature assumed) by dividing the interval time by the LPD and multiplying the resulting value by 100. The percent LPD contributed by each interval is accumulated and displayed interval-by-interval until 100% of the time in lag phase has elapsed as shown in the following formula:

$$\text{Total percent LPD} = \sum_{i=1}^N \frac{\text{interval time}}{\text{LPD}_i} \times 100$$

After lag phase is complete, interval accumulation is used to estimate subsequent growth, in log CFU. As shown in the following formula, the log CFU of growth is computed by multiplying GR (log CFU/minute) by either the time (minutes) remaining in the interval during which lag phase ended, or by the total time of the interval (for all intervals thereafter).

$$\text{Total Growth} = \sum_{i=1}^N \text{GR for interval}_i \times \text{interval time}_i$$

When temperature values entered by the user do not coincide with experimental temperatures used (2.8°C intervals from 10°C to 43.3°C), linear interpolation is performed between DMFit-derived LPD and GR values to obtain LPD and GR values for use in calculating the prediction.

## TESTING THE TOOL

The accuracy of THERM was tested, as described in Ingham et al,<sup>3</sup> and summarized here, in 20 inoculation studies with coarse-ground beef or poultry products. These products were inoculated, subjected to various short-term temperature-abuse regimes, and analyzed to determine pathogen populations at predetermined time points during the temperature abuse. A time/temperature history for either the product (4.5 kg chubs of coarse-ground beef) or the storage environment (poultry products) was also obtained in

each experiment to enter into THERM and obtain a growth prediction.

Coarse-ground beef in 4.5 kg sealed cylindrical packages (chubs) was obtained from a local wholesale distributor. Compositional and microbiological analyses were done as described above. To inoculate the chubs, 12 samples (25 g each) were removed by cutting incisions through the packaging material on the top half of the long axis of the chub. The 25 g samples were placed in small sampling bags, inoculated, and the inoculum was dispersed as described earlier. The bagged inoculated samples were placed back into the chubs just underneath the packaging and secured. For each coarse-ground beef experiment (Table 1, experiments 1 through 12), a temperature probe attached to a data logger was inserted just under the surface in the center of the chub to record time/temperature data that was then entered into THERM. Data points were selected by dividing the experiment time into 20 equal intervals, and determining the temperature at each of the times from the data logger output. Inoculated coarse-ground beef chubs were subjected to one of 3 temperature-abuse situations:

1. Pathogen contamination of refrigerated raw meat followed by short-term holding (3 hours to 6 hours) at room temperature or 35°C (experiments 1 through 4).
2. Thawing previously contaminated and frozen product at room temperature or 35°C (12 hours to 15 hours; experiments 5 through 8).
3. Holding previously contaminated, frozen, and thawed meat at room temperature or 35°C for 12 hours (experiments 9 through 12).

Sampling (one sample bag per inoculum) and pathogen enumeration were done as described earlier at predetermined times throughout each experiment. Frozen or frozen/thawed samples were spread-plated on Nutrient Agar and incubated at 35°C for one hour to encourage repair of freeze/thaw-induced injury. The Nutrient Agar was then overlaid with the appropriate selective medium (tempered at 48°C). Incubation then continued at 35°C for the usual 24 or 48 hour period.

For the poultry experiments (Table 1, experiments 13 through 20), fresh skinless chicken breasts and ground turkey meat from 2 different processors were obtained at a local retail store, stored frozen, and then thawed at 5°C before use. Frozen turkey scapula meat was

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obtained from a local wholesale distributor, stored frozen, and then thawed at 5°C before use. Both compositional and microbiological analyses were done as described above. Each type of poultry product was subdivided into small portions, inoculated as described earlier, and then exposed to 2 different temperature-abuse storage conditions. Storage treatments were either 13°C for 8 hours (experiments 13 through 16), mimicking a processing environment barely complying with USDA regulations,<sup>5</sup> or sequentially for 3 hours at 13°C, 3 hours at 21°C, and 4 hours at 30°C (experiments 17 through 20). The latter experiments mimicked an extreme loss of temperature control (eg, product inadvertently left on a loading

Table 1. Outline of temperature-abuse experiments with inoculated coarse-ground beef (GB), skinless chicken breasts (C), turkey scapula meat (T), ground turkey meat from plant A (GT-A) and ground turkey meat from plant B (GT-B).

Experiment	Product	Storage Temperatures	Holding Time
1	GB	RT*	340 minutes <sup>†</sup>
2	GB	RT*	360 minutes <sup>†</sup>
3	GB	35°C	180 minutes <sup>†</sup>
4	GB	35°C	240 minutes <sup>†</sup>
5	GB	-20°C/RT	15 hours <sup>†</sup>
6	GB	-20°C/RT	15 hours <sup>†</sup>
7	GB	-20°C/35°C	12 hours <sup>†</sup>
8	GB	-20°C/35°C	12 hours <sup>†</sup>
9	GB	-20°C/5°C/RT	12 hours <sup>††</sup>
10	GB	-20°C/5°C/RT	12 hours <sup>††</sup>
11	GB	-20°C/5°C/35°C	12 hours <sup>††</sup>
12	GB	-20°C/5°C/35°C	12 hours <sup>††</sup>
13	C	5°C/13°C	8 hours <sup>†</sup>
14	T	5°C/13°C	8 hours <sup>†</sup>
15	GT-A	5°C/13°C	8 hours <sup>†</sup>
16	GT-B	5°C/13°C	8 hours <sup>†</sup>
17	C	5°C/13°C/21°C/30°C	10 hours <sup>§</sup>
18	T	5°C/13°C/21°C/30°C	10 hours <sup>§</sup>
19	GT-A	5°C/13°C/21°C/30°C	10 hours <sup>§</sup>
20	GT-B	5°C/13°C/21°C/30°C	10 hours <sup>§</sup>

\*RT - room temperature ( $\approx 21^{\circ}\text{C}$ )

†Holding time at room temperature ( $\approx 21^{\circ}\text{C}$ ), 13°C, or 35°C. Initial sampling occurred on inoculation (for nonfrozen coarse-ground beef samples), when inoculated poultry products were moved to 13°C storage, or when frozen coarse-ground beef was removed from the freezer.

‡Sampling done once center of the meat mass reached 5°C and periodically during storage at RT or 35°C.

§Product held at 13°C for 3 hours, 21°C for 3 hours, and at 30°C for 4 hours. Initial sampling was done when product was moved to 13°C storage.

dock, cooler failure). Small pieces of meat were excised from the surface of the skinless chicken breasts and the turkey scapula meat, placed into a small sample bag and inoculated with 100  $\mu\text{L}$  of the appropriate inoculum, which was then distributed over the surface of each piece. Ground turkey meat (25 g) was weighed into a small sample bag, inoculated, and the inoculum was manually dispersed as described earlier. A data logger again was used to monitor storage temperature and provide data to enter into THERM. Data points were selected by dividing the experiment time into 20 equal intervals, and determining the temperature at each of the times from the data logger output. One sample of each product type was analyzed at 3 hours, 6 hours, and 8 hours (experiments 13 through 16) or at 3 hours, 6 hours, and 10 hours (experiments 17 through 20). Microbiological analyses were conducted as described earlier.

### STATISTICAL ANALYSIS

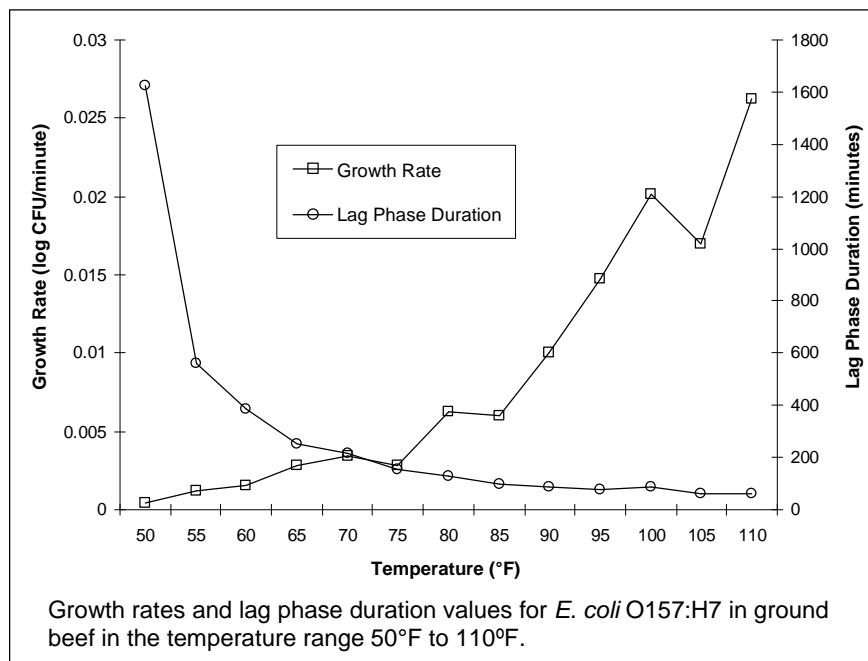
The paired t-test, with a 5% significance level was used to compare LPD and GR values for a given pathogen between products (ground pork, ground beef, ground turkey) and for a given product between pathogens (*E. coli* O157:H7, *Salmonella* serovars, and *S. aureus*). In experiments to test THERM, each log CFU value obtained in an experiment was subtracted from its corresponding time-zero value to obtain observed change in log CFU values. Time/temperature data from each experiment were entered into THERM to obtain predicted change in log CFU values. The observed values were compared to the predicted values using the paired t-test and regression analysis.

### RESULTS AND DISCUSSION

Preliminary experiments showed that the level of indigenous microorganisms had a significant effect on LPD values for *S. aureus* but not *E. coli* O157:H7 and *Salmonella* serovars, with larger *S. aureus* LPD values resulting when there were greater numbers of indigenous organisms. Therefore, in order to develop a conservative predictive tool, all development experiments used meat products which had what we considered to be low, but realistic, levels of background organisms—Aerobic Plate Count  $<3.5$  log CFU/g. This level was slightly lower than mean Aerobic Plate Count values reported in United States Department of Agriculture baseline surveys of ground beef, turkey, and chicken.<sup>6-8</sup>

The DMFit-derived R-squared value is a number from 0 to 1 that represents the relative predictive power of the model. The closer the R-squared value is to 1, the greater the model's accuracy. All R-squared values for each meat/pathogen combination were  $>0.70$  with 73%  $\geq 0.90$ . The only statistically significant ( $p<0.05$ ) differences in LPD or GR values were:

1. LPD values for *Salmonella* serovars were lower in beef than in pork.
2. LPD values for *S. aureus* were higher than those for *E. coli* O157:H7 and *Salmonella* serovars in beef, and higher than those for *E. coli* O157:H7 in pork.
3. GR values for *S. aureus* were lower than those for *E. coli* O157:H7 and *Salmonella* serovars in beef and turkey, respectively.



As expected, LPD decreased and GR increased as temperature increased to an optimum for growth (illustrated in the Figure).

As concluded in Ingham et al,<sup>3</sup> the THERM tool was accurate or fail-safe in predicting whether *E. coli* O157:H7, *Salmonella* serovars, and *S. aureus* grew in raw beef and poultry products during experiments designed to test the tool's performance. To reach this conclusion, we qualitatively evaluated the predicted and observed change in log CFU values, ie, described

predictions and observations as either "growth" or "no growth". We used the criteria of growth equals a change in log CFU $>0.3$  (more than one doubling) and no growth equals a change in log CFU $\leq 0.3$ . As shown in Table 2, the THERM tool accurately predicted whether growth would occur in 67%, 85%, and 95% of experiments involving *E. coli* O157, *Salmonella* serovars, and *S. aureus*, respectively. In all other experiments, THERM predicted pathogen growth when it was not observed experimentally, ie, made a fail-safe prediction. Notably, THERM never made a "fail-dangerous" prediction, ie, THERM never failed to predict growth when it was observed experimentally. In addition to comparing the predicted and observed change in log CFU values for each pathogen in each individual experiment, the paired t-test was used to compare predicted and observed change in log CFU values for each pathogen for all experiments combined. This analysis showed that the predicted changes in log CFU values were significantly higher than the observed change in log CFU values for *E. coli* O157:H7 ( $p=0.007$ ) and *Salmonella* serovars ( $p=0.02$ ). The R-squared values from regression analysis were 0.94 and 0.89, respectively for these 2 pathogens, indicating a very consistent relationship between predicted and observed values. The R-squared value for *S. aureus* was only 0.43, reflecting the divergence of predicted and observed values in poultry product experiments 17 through 20. The paired t-test analysis did not show any statistically significant difference between predicted and observed values for *S. aureus* for all experiments combined

( $p=0.49$ ), perhaps reflecting the inconsistent relationship between predicted and observed results. An additional way of testing the accuracy of THERM is to compare its pathogen growth predictions to experimental pathogen growth observed in experiments in other laboratories. For example, a recent study by Mann et al<sup>9</sup> suggested a critical limit for ground beef processors of "time in the processing area of 6 hours or less" with the processing area temperature defined as 22°C to 23°C. For 22.5°C storage of ground beef for 6 hours, with no additional

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Table 2. Accuracy of qualitative predictions (THERM\*) for growth ( $>0.3 \log \text{CFU}^\dagger$  increase) or no growth ( $\leq 0.3 \log \text{CFU}$  increase) of *Escherichia coli* O157:H7 (EC), *Salmonella* serovars (SALM), and *Staphylococcus aureus* (SA) in coarse-ground beef, on skinless chicken breasts and turkey scapula meat, or in ground turkey from plants A and B during storage at abusive temperatures.

Product & Experiment	EC growth $>0.3 \log \text{CFU}^\dagger$ ? + = yes - = no			SALM growth $>0.3 \log \text{CFU}^\dagger$ ? + = yes - = no			SA growth $>0.3 \log \text{CFU}^\dagger$ ? + = yes - = no		
	Observed	Predicted	Accuracy	Observed	Predicted	Accuracy	Observed	Predicted	Accuracy
<b>Coarse-Ground Beef</b>									
1	-	-	Acc <sup>‡</sup>	-	-	Acc	-	-	Acc
2	-	+	F-S <sup>§</sup>	-	+	F-S	-	-	Acc
3	-	-	Acc	-	-	Acc	-	-	Acc
4	-	+	F-S	+	+	Acc	-	-	Acc
5	-	+	F-S	-	+	F-S	-	-	Acc
6	-	-	Acc	-	-	Acc	-	-	Acc
7	+	+	Acc	-	+	F-S	-	-	Acc
8	+	+	Acc	+	+	Acc	-	+	F-S
9	-	+	F-S	-	-	Acc	-	-	Acc
10	+	+	Acc	+	+	Acc	-	-	Acc
11	+	+	Acc	+	+	Acc	+	+	Acc
12	+	+	Acc	+	+	Acc	+	+	Acc
<b>Ground Beef Totals</b>	67% Acc 33% F-S			75% Acc 25% F-S			91% Acc 9% F-S		
<b>Chicken Breast</b>									
13	NT <sup>**</sup>	NT	NT	-	-	Acc	-	-	Acc
17	NT	NT	NT	+	+	Acc	+	+	Acc
<b>Turkey Scapula</b>									
14	NT	NT	NT	-	-	Acc	-	-	Acc
18	NT	NT	NT	+	+	Acc	+	+	Acc
<b>Ground Turkey A</b>									
15	NT	NT	NT	-	-	Acc	-	-	Acc
19	NT	NT	NT	+	+	Acc	+	+	Acc
<b>Ground Turkey B</b>									
16	NT	NT	NT	-	-	Acc	-	-	Acc
20	NT	NT	NT	+	+	Acc	+	+	Acc
<b>Total for Poultry</b>				100% Acc			100% Acc		
<b>Overall Total</b>	67% Acc 33% F-S			85% Acc 15% F-S			95% Acc 5% F-S		

\*Temperature History Evaluation of Raw Meats

<sup>†</sup>Colony Forming Unit

<sup>‡</sup>Accurate

<sup>§</sup>Fail-safe, ie, predicted growth  $>0.3 \log \text{CFU}$ , and observed growth  $\leq 0.3 \log \text{CFU}$

<sup>\*\*</sup>Not tested

warm-up and cool-down times (the same conditions as laid out by Mann et al<sup>9</sup>), the THERM tool predicted a 0.54 log CFU increase of *E. coli* O157:H7. The experimentally determined change in log CFU value was 0.49, indicating good agreement between THERM and the inoculation study. With longer room-temperature incubation, Mann et al<sup>9</sup> observed a change in log CFU values (rounded) of 1.0, 1.4, and 1.8 for *E. coli* O157:H7 stored for 8 hours, 10 hours, and 12 hours, respectively. The corresponding changes in log CFU values predicted by THERM for these times were 0.9, 1.3, and 1.7. For ground beef stored at 10°C, Mann et al<sup>9</sup> observed changes in log CFU values of approximately 0.1, 0.1, 0.2, 0.4, 0.8, and 1.0 at 4 hours, 8 hours, 12 hours, 24 hours, 48 hours, and 72 hours, respectively. The THERM tool predicted no growth of *E. coli* O157:H7 through 27 hours, with changes in log CFU values of 0.5 at 48 hours and 1.1 at 72 hours. Using the qualitative approach discussed earlier, we conclude that the THERM tool predictions are consistent with those from the Mann et al study.<sup>9</sup>

Experimentally determined critical limits, as in the first Mann et al<sup>9</sup> example above, expressed in maximum safe time at a given temperature, are useful for processors, so critical limit tables were developed for each meat type based on the shortest LPD time of the 3 pathogens. Table 3 was used to evaluate several out-of-temperature situations reported in the US Army Veterinary Command's Installation Support Plan database.<sup>10</sup> In one example, raw beef and poultry items were exposed to an out-of-temperature situation for 7 hours and had reached internal temperatures of 50°F. Based on our critical limit table, raw beef can be safely exposed to this temperature for up to 27 hours and raw poultry up to 22.5 hours. Another example was a reported out-of-temperature situation involving a raw-meats display case (meat type not reported) in which items were temperature-abused for 5 hours and reached a temperature of 57°F. The THERM critical limit table reports safe exposure times at this

Table 3. Critical limit table expressed in maximum safe time, in hours and minutes, at a given temperature for pork, beef, and poultry

Temperature (°F)	Critical Limit (hours:minutes)		
	Pork	Beef	Poultry
50	54:45	27:00	22:30
55	17:30	9:00	14:45
60	9:00	6:00	13:45
65	8:15	3:45	8:15
70	5:45	3:30	4:45
75	4:15	2:30	3:00
80	4:15	2:00	3:00
85	1:30	1:45	2:00
90	1:30	1:30	2:00
95	1:30	1:15	2:00
100	1:30	1:15	1:30
105	0:45	1:00	0:45
110	0:45	1:00	0:45

temperature of 9 hours, 6 hours, and more than 13 hours for pork, beef, and poultry respectively. When several time and temperature measurements are available from a refrigeration failure situation, a more specific THERM prediction is possible. For example, in a recently reported out-of-temperature situation, internal product temperatures at 2 hours, 4 hours, 8 hours, and 12 hours were 42°F, 48°F, 60°F, and 38°F. For all meat types THERM predicted that 67% or less of LPD elapsed for *E. coli* O157:H7 and 58% or less of LPD elapsed for *Salmonella* serovars. Because no *S. aureus* growth occurred during 240-hour experiments at temperatures below 60°F in our isothermal studies, THERM did not predict

any LPD elapsing for *S. aureus* during this out-of-temperature situation.

One conservative feature of THERM is that it does not account for a variety of inhibitory ingredients (eg, fat, sodium chloride, sodium nitrite, liquid smoke) or inhibitory processing conditions (eg, dry-curing, cold-smoking, or drying) to which pathogens or competing microorganisms may be exposed during temperature abuse of raw meat products. For example, in 2 independent industry challenge studies involving partially cooked bacon and finished biltong and droëwors\* conducted by our laboratory,<sup>11,12</sup> THERM always predicted growth of all 3 pathogens when experimental time/temperature data were entered. Yet, no growth of inoculated pathogens was observed in any of the trials. To make the most accurate predictions for critical limit validation or process

\*Biltong and droëwors are 2 shelf-stable, ready-to-eat, dried beef products developed in South Africa. Traditionally these products were made by drying under ambient conditions. To make biltong, beef strips are seasoned (high-salt) and dried. To make droëwors, small pieces of beef are obtained from trimming and/or grinding, seasoned (high-salt), stuffed into casings, and dried. In essence, these two products could be viewed as very thick versions of whole-muscle and ground-and-formed beef jerky, made without elevated heat.

## Using Predictive Microbiology to Evaluate Risk and Reduce Economic Losses Associated with Raw Meats and Poultry Exposed to Temperature Abuse

deviation decision making for these products, additional versions of THERM would have to be developed using meats containing representative amounts of salt, nitrite, and/or other inhibitory compounds. Other researchers have attempted to account for inhibitors of microbial growth and have modeled the temperature, pH, and water activity conditions at the growth/no growth boundary for *Salmonella* Typhimurium in laboratory media.<sup>13</sup> However, analogous work with meat systems has not been published. We are currently working on a version of THERM using a standardized pork-based bratwurst batter. Another restriction of THERM is its limited temperature range. However, growth at temperatures below the THERM lower limits would likely have little effect on the accuracy of THERM predictions because of the extremely long lag phase duration values expected at such low temperatures. However, THERM growth predictions could be erroneously low if growth occurred at temperatures above the 43.3°C upper limit of THERM. Furthermore, at even higher temperatures, such as those employed in slow-cooking, pathogens will begin to die. We are currently studying the expansion of THERM beyond the 43.3°C upper limit. However, many physiological and environmental factors must first be considered before developing a tool that predicts pathogen behavior at the upper growth/no growth temperature boundary, and when temperature is high enough to cause cell death.

## CONCLUSIONS

THERM is an effective prototype tool for qualitatively predicting pathogen behavior in raw meat and poultry. Its application in Department of Defense temperature deviation evaluation could reduce economic losses associated with temperature-abused meats without jeopardizing the health of armed forces personnel. Ongoing extensions of the THERM prototype will enhance its applicability. The current downloadable version of THERM, as well as a web-based beta-version, may be found on the website, <http://www.meathaccp.wisc.edu>.

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# The US Army Veterinary Corps Reserve Component

CPT(P) Christopher A. Young, VC, USA

The US Army Veterinary Corps Reserve Component (VC RC) is composed of Citizen Soldiers who are leaders and experts in the civilian veterinary medical community, as well as leaders in their communities. This article briefly explains the Veterinary Corps' mission as defined by Army doctrine and demonstrates the Veterinary Corps' role in Force Health Protection as described in the Force Health Protection Capstone

Document,<sup>1</sup> prepared by the Medical Readiness Division, J-4, Joint Forces Command. The diverse composition of the VC RC is featured through highlighting the military education, civilian education, military assignments, and deployments of its Soldiers.

An overview of the responsibilities of US Army Veterinarians is stated in *Field Manual 4-02.18*<sup>2</sup>:

## 1-3. Veterinary Concept of Operations

Veterinary services function in three broad categories. These categories include:

- Food safety, food defense, and quality assurance
- Veterinary medical care
- Veterinary preventive medicine

- a. Food Safety, Food Defense, and Quality Assurance Services. Food safety includes hygiene and sanitation, defense, and quality assurance services as a primary component of preventing disease and nonbattle injury (DNBI) within an AO [area of operations].
- b. Veterinary Medical Care. Level I and II veterinary care for MWDs [military working dog] includes emergency treatment, stabilization, and evacuation. There is no Level IV veterinary care and Level V veterinary care is found in CONUS [continental United States] at the DoD MWD Center. Level III veterinary medical and animal hospital care is provided by the MDVM. Level III veterinary hospital care includes comprehensive veterinary medical and surgical animal hospital care. The levels of veterinary medical care and the number of veterinary detachments deployed to an AO are determined by mission, enemy, terrain and weather, troops and support available, time available, and civil considerations (METT-TC). At all levels of veterinary medical care, surveillance, prevention, and control programs for diseases common to both animal and man are implemented. The senior veterinary staff officer provides advice and guidance on these threats to the medical commanders and command surgeons.
- c. Veterinary Preventive Medicine.
  - Support prevention and control programs to protect soldiers from foodborne diseases.
  - Evaluate zoonotic disease data collected in the AO and advise PVNTMED [preventive medicine] elements and higher headquarters on potential hazard(s) to humans.
  - Establish animal disease prevention and control programs to protect soldiers and their families and other DoD and Allied personnel from zoonotic diseases.
  - Assess the presence of animal diseases that may impact the CONUS agriculture system if contaminated equipment or personnel are allowed to redeploy.
  - Perform investigations of unexplained animal deaths to include livestock and wildlife.

The military veterinarian's doctrinal mission is described above, but Reserve Component Veterinary Corps officers (VCO) are not limited to assignments in Medical Detachment (Veterinary Service) units. VCOs may be assigned to Civil Affairs units where they may participate in agricultural and/or preventive medicine teams. Veterinarians participating in these missions work on a wide range of activities from developing infrastructure to designing and implementing veterinary preventive medicine programs for host nation livestock. Another potential VCO assignment is the newly created Medical Readiness Training Command. Assigned veterinarians are working to integrate Active Army and Reserve Component training opportunities and to appropriately utilize the Medical Training Brigades and RTS Meds. VCOs can accept assignments as Drilling Individual Mobilization Augmentees, who serve as resources for both District Veterinary and Regional Veterinary Commands. There are several colonel level assignments within the structure, including medical brigades, the Army Medical Command, and Office of The Surgeon General. There are currently 12 Veterinary Corps officers serving in the National Guard. Regardless of assignment, the small family of approximately 173 Reserve Component VCOs is a tight-knit group that represents tremendous experience in many facets of veterinary medicine.

The relevance of the veterinary mission is as strong today as it was at the origin of the Army Veterinary Corps, when the horse played a fundamental role in the logistics apparatus of the military. As such, animal medicine was the key mission. Eventually, however, the Veterinary Corps' role grew to encompass procurement of subsistence. The focus on food safety evolved from the findings by the military that the foodstuffs procured for the soldiers fighting the Spanish-American War were substandard and filled with vermin and contaminants. In addition, Upton Sinclair's novel *The Jungle*,<sup>3</sup> with its exposé of the horrendous conditions in the meat packing industry, led to congressional passage of both the Pure Food and Drug Act and the Meat Inspection Act in 1906. The Veterinary Corps mandate expanded to ensure that government food procurement contracts were fulfilled with integrity, as is still the case today. In fact, today the Veterinary Corps is the Department of Defense Executive Agency for fulfilling the veterinary mission for all branches of the Armed Forces, plus supporting numerous executive branch agencies. Further, the

Veterinary Corps role in force health protection is a critical mission. In 2006, the Medical Readiness Division (J4) of the US Joint Staff prepared the Force Health Protection Capstone Document.<sup>1</sup> The document prioritizes 10 critical success factors. The top priority is in the category of **Healthy and Fit Force**, of which item one is:

#### **Occupational and environmental health**

- Identify, evaluate, and control potential chemical, biological, and physical hazards.

The fourth listed priority is again in the category of **Healthy and Fit Force**. The selected items are relevant to military veterinary medicine:

#### **Injury/disease prevention. Goal: in prevalence/ incidence**

- Identify preventable injuries and disease affecting mission readiness.
- Establish standards for occurrence rates and acceptable behaviors.
- Develop prevention strategies.

The final category relevant to the veterinary mission is **Surveillance**. The following items are impacted by military veterinarians:

#### **Develop a joint comprehensive standard health surveillance system**

- Environmental/occupational capability
- DNBI capability
- Operational casualties capability
- Linkages to personnel exposure (location and duration information)
- Seamless garrison/field capability

With the priorities of the Capstone Document<sup>2</sup> as a backdrop, consider the context of a deployment. No medical professional is better prepared to address the complex interface between the Soldier, wildlife, domestic animals, and the environment than veterinary specialists. Zoonotic agents make up 65% or more of the agents that cause infectious disease. Many of these are foreign animal diseases, which, by definition, are caused by agents that do not occur in CONUS. To address these concerns, a majority of the officers in the Veterinary Corps are Preventive Veterinary Medicine specialists. Further, many officers are trained Foreign

## ***The US Army Veterinary Corps Reserve Component***

Animal Disease Diagnostician, veterinarians that have specialized training in diagnosing the clinical signs and gross pathology of foreign animal diseases.

The Reserve Component has the additional benefit of bringing the civilian job skills and experience of its members to bear when deployed. Such value-added contribution by the Reserve Component veterinarians is especially profound. As mentioned earlier, there are approximately 173 RC VCOs. Indicative of the level of professionalism and skill represented within the ranks of those officers are the number of board certifications and diversity of specialties, displayed in the Table.

<b>Specialty certifications of US Army Reserve Veterinary Corps Officers</b>	
<b>Board of Certification</b>	<b>Number Certified</b>
Veterinary Pathology	10
Laboratory Animal Medicine	4
Veterinary Preventive Medicine	8
Veterinary Internal Medicine	5
Veterinary Practitioner	1
American Board of Toxicology	1
Veterinary Surgery	1

Fifteen officers hold post-doctoral degrees, including 11 master's and 4 PhDs. At the writing of this article, 4 RC VCOs are completing residency programs in theriogenology, pathology, internal medicine, and anesthesiology, respectively. Degrees and board certifications are important, but there are other metrics that demonstrate the exceptional caliber of the officers in the Veterinary Corps Reserve Component, whose ranks include:

- 2003 Kentucky Veterinarian of the Year
- 2002 Oklahoma Veterinarian of the Year
- 2003 Texas Specialty Veterinarian of the Year
- 2003 Kansas Veterinarian of the Year

- 2006 Washington State University College of Veterinary Medicine Outstanding Service Award winner
- One of the Auburn University College of Veterinary Medicine Class of 1994 Young Achievers.

Such honors speak volumes about the quality of the officers. The RC VCOs are engaged in a wide range of civilian veterinary careers, including epidemiology, laboratory pathology, lab animal medicine, colleges of veterinary medicine, mixed animal practice, large animal practice, small animal practice, specialty practices, among others. RC VCOs hold positions of leadership in organized veterinary medicine at both state and national levels. The officers are involved at the community level with hospital boards, religious organizations, charities, local government offices, Boy Scouts, Cub Scouts, sports teams, and more.

The following characterization of excellence truly exemplifies the Veterinary Corps Reserve Component:

Excellence is the result of caring more than others think is wise, risking more than others think is safe, dreaming more than others think is practical, and expecting more than others think is possible. (anonymous)

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# ***Special Operations Forces Veterinary Personnel***

COL Robert Vogelsang, VC, USA

There is no Additional Skill Identifier or Area of Concentration designating US Army Veterinary Corps Soldiers as “Special Operations.” However, members of the Corps currently serve in and support Special Operations Forces (SOF) units. Veterinary Corps officers and enlisted animal technicians have been part of SOF since at least World War II. Due to the heavy use of pack animals, veterinary personnel were part of Merrill’s Marauders and the MARS Task Force, employed in the China-Burma-India theater of operations. The MARS Task Force included the reorganized remainder of the Marauders (redesignated the 475th Infantry Regiment), the 124th Cavalry Regiment, as well as some Quartermaster Pack Troops. The 475th Infantry Regiment was redesignated the 75th Infantry Regiment in 1954, the direct ancestor of which is the now the 75th Ranger Regiment.

Army Veterinary Corps involvement in Army Special Forces goes back to at least the early 1960s when veterinarians were assigned to Civil Affairs Groups in Okinawa (97th) and Panama (3rd). At that time, however, even though veterinarians were going through the Special Forces Qualification Course, they were not assigned to Special Forces Groups (Airborne). Such assignment did not begin until the mid-1960s. The association of Veterinary Corps personnel with Civil Affairs and Special Forces units continues to the present day.

Veterinarians in Special Forces Groups (SFG) assist in planning and executing preventive medicine tasks to preserve the health of the Group, and participate in veterinary civic action programs, also known as “hearts and minds,” during exercises and deployments. Group veterinarians also assist in the training of Special Forces Medical Sergeants (Army military occupational specialty [MOS] 18D) through activities such as didactic and hands-on instruction in small and large animal emergency treatment, food safety, zoonotic and foreign animal disease recognition, pack animal operations, animal husbandry and general veterinary care, and field slaughter and carcass

evaluation. There are 5 Active Army and 2 National Guard SFGs.

Although the Army Reserve Civil Affairs units were taken out of US Army Special Operations Command (USASOC) in October 2006 and placed under US Army Reserve Command, the Active Army 95th Civil Affairs Brigade remains under USASOC. The 95th and its 4 subordinate battalions each have a Veterinary Corps officer on their Table of Organization and Equipment.

The mission of the Civil Affairs (CA) veterinarian is to work with indigenous military assets and allied, coalition, or foreign government agencies. They assist in planning and executing population and resource control, civic action, and other security development and stability programs. During military and paramilitary operations, they assist in planning and executing civic action, humanitarian assistance, and other programs designed to expand the host nation government’s legitimacy. The CA veterinarian also provides estimates and data on the resources essential to build an effective infrastructure for civil health and agricultural administration operations.

The CA veterinarian offers technical advice to the commander on issues of agricultural production systems; effects of large-scale, cross-border livestock movements; effects from outbreaks of endemic and foreign animal diseases; and cooperation with non-governmental organizations.

Additionally, veterinarians are part of the Special Operations Sustainment Brigade and the John F. Kennedy Special Warfare Center and School. Animal Care (MOS 68T) noncommissioned officers (NCO) are also authorized and assigned to the school. All SOF positions require parachute qualification.

There are veterinarians on the Surgeon’s staff at both USASOC and US Special Operations Command (USSOCOM). Though all SOF veterinary personnel currently serve in Army units except USSOCOM, a Veterinary Corps officer and Animal Care NCO are

## ***Special Operations Forces Veterinary Personnel***

expected to become authorized and assigned to the US Marine Corps Force Special Operations Command (MARSOC) in CY08. These personnel will care for Military Working Dogs (MWD) assigned to MARSOC as well as perform a role similar to that of the SFG veterinarian when the unit is deployed. The 75<sup>th</sup> Ranger Regiment has indicated an interest in obtaining a veterinary support capability which, if approved, would authorize assignment of one Veterinary Corps officer and at least one Animal Care NCO.

SOF veterinary personnel have been awarded or qualified for the Combat Action Badge, as Special Operations Combat Diver, and Pathfinder. A number are Senior and Master Parachutists, and some have foreign jump wings. One National Guard SFG veterinarian is a member of the World War II Airborne Demonstration Team, a civilian reenactment group.

All SFG veterinarians and most of the other SOF personnel have been deployed in support of Operations Iraqi Freedom and Enduring Freedom. Due to the sometimes remote locations in which SOF operates, the unit Veterinary Corps officer may be the only veterinarian in a particular area. As such, they will work with host nation public health personnel to improve local capabilities. SOF veterinarians have responded to suspected animal diseases, and, specifically in Afghanistan, worked with a local veterinarian to collect and submit samples to the Ministry of Agriculture and Animal Health for diagnosis. Also, at the request of the Afghan agricultural ministry, a SOF veterinarian gave lectures on Highly Pathogenic Avian Influenza (HPAI) and other animal diseases to a group of local veterinarians. Shortly thereafter, the first reported case of HPAI occurred in Afghanistan and a Group Veterinarian assisted in the collection and distribution of essential veterinary medical supplies to veterinarians in outlying areas. The Group Veterinarian helped to educate personnel working at veterinary diagnostic labs in the proper application and use of the Brucellosis card tests that were provided by the US Department of Agriculture through the efforts of other Army veterinarians deployed to Afghanistan.

The Group Veterinarians participated in numerous veterinary civic action programs throughout the deployments. Several thousand animals were vaccinated and dewormed during these missions, improving relations with local livestock owners and

government officials. Whenever possible, local veterinarians were encouraged to participate in these missions to help build a trust in the local infrastructure and services available. This interaction also provided excellent opportunities for the sharing of knowledge between American and local veterinarians.

To provide alternative modes of transportation in areas where vehicular travel was not practical, several firebases obtained horses, mules, and donkeys from local stock. Group Veterinarians assisted in the purchase of and care for these animals, one VCO performed castrations on several stallions and assisted during the foaling season when 6 mares gave birth on one firebase.

Group Veterinarians conducted spay and neuter surgeries on animals owned by local nationals that were in close proximity to coalition personnel. These animals were also vaccinated against rabies and dewormed to reduce the risk to human health in those areas.

Group Veterinarians worked with Preventive Medicine Officers to ensure that dining facilities were properly maintained and that the risk of food-borne illness was minimized at bases throughout Afghanistan. One Group Veterinarian also assisted in human trauma cases at a Forward Surgical Element's local trauma center in southern Afghanistan.

Veterinary support of special operations forces has grown significantly in the last decade and will likely continue to do so within the current operational climate. Despite the fact that there is no veterinary Area of Concentration for special operations, there are now a sufficient number of higher ranking positions that may allow a Veterinary Corps officer to have a type of career progression within the Special Operations environment. SOF assignments are generally considered one of the best and most rewarding tours of duty within the career of a Veterinary Corps officer. Remember, there's no greater threat than an Airborne Vet!

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# ***Stabilization And Reconstruction Operations: The Role Of The US Army Veterinary Corps***

LTC John C. Smith, VC, USA

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The events of September 11, 2001, taught us that weak states, like Afghanistan, can pose as great a danger to our national interests as strong states. Poverty does not make poor people into terrorists and murderers. Yet poverty, weak institutions, and corruption can make weak states vulnerable to terrorist networks and drug cartels within their borders.

President George W. Bush

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## **ABSTRACT**

Stabilization and reconstruction operations in failed or failing states are vital to US security interests. These operations require a bottom-up approach, focusing on the population as the strategic center of gravity. This bottom-up approach must address the population's basic needs, as defined by Dr Abraham Maslow's hierarchy of needs, and provide a long-term means of self-sufficiency, rather than creating an "aid dependent economy." Focusing operations on agricultural projects provides relief from donor dependency, stimulates economic growth, and thwarts the power of spoilers.

US Army Veterinary Corps personnel provide essential services ensuring the procurement of safe and wholesome subsistence and provision of medical care to government-owned animals. Veterinary Corps officers are also uniquely qualified to design and implement agricultural stabilization and reconstruction programs in conjunction with host-state ministries and agencies across the full range of military operations. Early, sustained engagement by veterinarians stimulates agricultural productivity, improves animal and human health, directly supports the population's hierarchy of needs on all levels, and accelerates stabilization operations by reducing the population's susceptibility to spoilers.

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Stabilization and reconstruction operations in failed or failing states are vital to US security interests. Diminishing the conditions that permit terrorism to flourish and denying terrorists safe haven in failed states are among the objectives of the National Military Strategy to protect the United States.<sup>1</sup> In his foreword to the 2005 National Defense Strategy, Defense Secretary Rumsfeld emphasized that the United States must take actions to influence events before they become more dangerous and less manageable. He also suggested that we must transform how we think about security to achieve the favorable security conditions required to protect the homeland and US interests around the world.<sup>2</sup>

## **CAUSES OF STATE INSTABILITY**

Economic distress, including food insecurity, urbanization, refugee and displaced persons movements, incapacitated and/or corrupt governments, infectious diseases, and socioeconomic disparity challenge the security and survival of individuals and communities, and are primary destabilizing factors leading to weakened and failed states.<sup>3,5</sup> These long-term factors are closely interrelated, and the presence of multiple factors results in synergistic negative effects on the population, creating or exacerbating state instability.<sup>3</sup> Early identification, intervention, and improvement in the state's agricultural sector can

reduce the possibility of a humanitarian crisis or mounting insurgency.<sup>7</sup>

Between 70% and 75% of the world's poor live in rural areas and earn their income directly or indirectly from agriculture and agriculture-related activities.<sup>6,7</sup> The majority of these poor agriculturalists reside in weak states and are highly subject to insurgent and terrorist influences. In developing states, the performance of the agricultural sector determines the state's overall economic growth, expansion of trade, and income-earning opportunities.<sup>6,7</sup> Improving agricultural productivity reduces poverty and stimulates economic growth in all sectors. In fragile states, the assistance objectives of the United States must focus on stabilization, recovery, and reform.<sup>8</sup> A goal of agricultural programs is to tie the population to their homes and land where they are interdependent on the land for their livelihood and cannot afford to leave it. Food donations counteract this concept, requiring people to leave the farm and increasing their susceptibility to insurgents and terrorists.

## **STABILITY AND RECONSTRUCTION OPERATIONS**

During stability and reconstruction operations, the goal is not to return the state to "status quo antebellum," but to improve the conditions for the population in order to eliminate the root causes of the instability.<sup>9(p2)</sup> During stability and reconstruction operations, there will be "spoilers" to that effort, be they insurgents, terrorists, religious or political factions, drug cartels, warlords, or individuals seeking to benefit themselves or their particular cause.<sup>9(pp11-15)</sup> Spoilers use the state's instability to further their causes and have little to gain should the needs of the population be met. As individual needs are met and state stability improves, the spoilers' active and passive support within the population providing recruits, supplies, and safe haven is diminished.<sup>10(pp16-20)</sup> Combat operations are focused on defeating the enemy strategic center of gravity. The same objective applies to stabilization and reconstruction operations. Current methods of stabilization and reconstruction are focused at the state level, working to improve the state as a whole, with wide-sweeping programs and projects. States cannot be stabilized from the top down, the center of gravity for stabilization and reconstruction operations is the state's population. State stabilization requires a sound foundation to build upon, and that foundation is a population that has its basic needs met and therefore can concentrate on the greater issues of state.<sup>3,4</sup>

## **MASLOW'S HIERARCHY OF NEEDS**

Dr Abraham Maslow, during the 1940s and 1950s, developed a theory of human motivation and advocated a personal hierarchy of needs. His theories generally have been accepted, with some criticism, and continue to be used as a basis of human motivational theory yet today. Many scholars have enhanced and added to Maslow's hierarchical needs concept over the years, but his basic building blocks remain steadfast. Maslow believed that humans are motivated, positively or negatively, by their unsatisfied needs and until the hierarchy's lower needs are satiated, the higher needs are unfulfilling and immaterial. Maslow considered humans to be generally nonviolent, trustworthy, self-protecting, and self-governing. People who are unable to meet their basic needs are motivated into actions to achieve fulfillment; their standards and morals may give way to survival instincts.<sup>11</sup> While many examples of deviant behavior exist in all societies, they are the exception rather than the norm. Rational individuals steal, lie, or murder not because they find pleasure in these activities, but because they have deficiencies in their basic needs. Maslow also identified conditions that are prerequisites for the basic needs to be satisfied. These conditions include the freedoms of speech, expression, and self-defense. Deficiencies in the individual basic needs must be satisfied before a person can begin to act unselfishly and be a fully productive member of society. Needs deficiencies also may occur on a society-wide level. Thus, when many people are hungry they look for relief outside their family, clan, or community. When this happens, the region is highly susceptible to influences from spoilers, whether or not they can truly affect the plight of the individuals. A strong leader's promises of a better life provide the population hope and allow the leader to gain influence over them.

Dr Maslow espoused that individual's needs are centered on 5 primary areas: biological and physiological needs, safety needs, belongingness and love needs, esteem needs, and the need for self-actualization.<sup>12-16</sup>

### **BIOLOGICAL AND PHYSIOLOGICAL**

Biological and physiological needs represent the most basic needs of an individual, and the population as a whole. At the nucleus of these needs are those that

keep an individual alive: food, water, and shelter from the elements. Until these needs are met, individuals cannot move to the higher levels of needs. Maslow hypothesized that even with the higher needs met, individuals cannot overcome their concerns for the lower-level needs. Military forces support host states in providing security to their populations and often facilitate the delivery of American and internationally donated humanitarian aid. However, this aid does not adequately address the basic need because it fails to provide the population the ability to meet biological and physiological needs for themselves. An often-used Chinese proverb is applicable to these situations:

Give a man a fish; you have fed him for today. Teach a man how to fish; and you have fed him for a lifetime.<sup>17</sup>

Food is an indispensable commodity even in the poorest of states, and the lack of food creates tension between the rich and the poor both within a state and among states.<sup>18</sup>

Support for struggling states in their efforts to develop sound agricultural programs reduces the threat of local and regional food crises, and creates the germinal conditions for continued economic growth—at a reasonable pace. As the 2002 US National Defense Strategy states:

Decades of massive development assistance have failed to spur economic growth in the poorest countries. Worse, development aid has often served to prop up failed policies, relieving the pressure for reform and perpetuating misery. Results of aid are typically measured in dollars spent by donors, not in the rates of growth and poverty reduction achieved by recipients. These are the indicators of a failed strategy.<sup>19(p21)</sup>

States, and their populations, become dependent and develop “aid economies” rather than developing their own agricultural or industrial capabilities.<sup>11</sup> Aid economies do provide jobs within the transportation, warehousing, distribution, and related fields, but these same jobs are available within an agricultural economy and are less subject to the abuse, graft, and economic inflation seen in aid economies.<sup>11</sup> Developmental assistance has focused on rapid transformation of poor states to bring them up to Western standards. What poor states need are structured programs of progress that likely will take years to fully mature; they need to be able to ride a bicycle before they can ride a Harley.<sup>20</sup>

## SAFETY

Safety needs is the second tier requirement that includes security; stability; protection; freedom from fear, anxiety, and chaos; and a desire for structure with law and order. Within safety needs, the ability to safely access the required biological and physiological needs may become a primary driving force to the individual or population. Security operations are a natural mission for US military forces during stabilization and reconstruction operations. However, without the population’s biological and physiological needs being met, they remain discontent and subject to spoiler influences.

During the Vietnam War, the Marine Corps conducted Operation Golden Fleece, designed to sever the Viet Cong from their source of food in the south.<sup>10(pp109-110)</sup> Marines provided security to Vietnamese villages and surrounding farmland during harvest season, allowing farmers to safely harvest and sell their crops without “taxation” by the Viet Cong. This program was successful on several Maslow levels. Besides the obvious biological and security needs, it provided the Vietnamese self-esteem in their ability to grow and harvest their own crops, maintained their sense of belonging by allowing them to remain in their homes and villages, and may have provided self-actualization. Another less successful US security program directly affecting agriculture in Vietnam was Operation Ranch Hand.<sup>10(pp109-110)</sup> This was a defoliation program with the intent of denying cover and concealment to the insurgents and thus reducing access to local Vietnamese villages and crops. While Operation Golden Fleece improved the situation for the local population, Operation Ranch Hand deteriorated it. Frequently, the application drift of Agent Orange killed the crops it was intended to protect. The loss of crop production alienated the local population and created strong resentment of US forces, which in turn strengthened the local support to the Viet Cong, as they were only demanding taxation, not the loss of an entire harvest.

## BELONGINGNESS AND LOVE

The third order of needs are the belongingness and love needs. These needs may be met with a sense of neighborhood, clan, or community belonging. People require a sense of belonging to their roots and origin where they can find security and stability. Refugees and displaced persons residing in artificial

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communities or fleeing into urban population centers lack that sense of belonging and easily may be enticed by spoilers' rhetoric to join them to achieve belongingness. Maintaining the population in rural areas by increasing their ability to produce their own subsistence, and then increasing production in order for them to create income reduces urbanization and overcrowding in the state's cities where they are less able to find work and are more susceptible to spoilers.<sup>21(pp217-218,227)</sup> Urbanization worsens environmental problems such as air and water pollution and creates a nidus for epidemics of infectious diseases, which may expand into international pandemics.<sup>22</sup> Land ownership provides a sense of belongingness, and the lack of land ownership often is among the root causes of civil unrest and insurgency, as was seen in the Philippines following World War II and recently in southern Africa. Land ownership provides individuals and family units a means of providing for their biological and physiological needs, gives them a sense of security and belongingness, and allows them to garner self-esteem and possibly achieve self-actualization. In the Philippines, granting land ownership was a successful method of disarming the insurgents and returning them to productive members of society.<sup>10(pp75-82)</sup> Trading guns for land ownership can be a successful method to support individuals in their quest to meet their basic needs and for the state to increase its influence and increase the local and national economic base.

### **ESTEEM**

Esteem needs are met by an individual's sense of self-esteem and self-respect along with the esteem derived from others in the form of respect, reputation, prestige, and praise. A man who cannot feed his family loses self-respect when standing in a humanitarian rations line, dependent on the mercy and goodwill of others. The dwindling self-respect leads to feelings of lowered self-worth and self-confidence. When individuals feel inadequate they are increasingly susceptible to the influence of spoilers promising them fame, glory, or status. Spoilers provide individuals with a sense of improvement in their self-worth, strength, and capability to improve their usefulness. Spoilers often have greater access to humanitarian aid shipments through coercion, theft, or graft and thus enable their followers to better provide for their families' needs. Frequently, international donations of subsistence fall prey to the black market and profiteers, who often are

insurgents or supporters of the insurgency, or to governmental corruption. In either case, the citizen does not gain from these humanitarian shipments. Only when the subsistence can be produced locally and sold openly on local free markets can the basic needs of the individual citizen be addressed.

### **SELF-ACTUALIZATION**

At the top of Maslow's hierarchical pyramid is self-actualization. Self-actualization is an individual's need to do what he strongly desires to do and is fitted to do, "what a man can be, he must be."<sup>23</sup> An individual cannot achieve self-actualization until the 4 previous needs have been fulfilled. A carpenter may find great satisfaction in his abilities and quality of work, but if he is hungry and afraid for his life and that of his family, he will not achieve self-actualization.

In order to deflate the power and influence of the spoilers, the population's basic needs must be met. It may require coordinated psychological operations efforts to bring the population to a solid realization that it is their basic needs that they desire, not the spoilers' rhetoric of meeting their "pie-in-the-sky" dreams. In many unstable states, international and nongovernmental organizations are trying to "improve the plight" of the citizens by bringing them into the modern world and providing them with all of the modern conveniences of the western world. Where concentration is really needed is on the individuals' and communities' first 4 basic needs. When the population is capable of meeting and maintaining those 4 needs, they will themselves develop into the modern world by seeking self-actualization.

### **US NATIONAL SECURITY STRATEGY**

Among the goals of the US national security strategy are championing aspirations for human dignity and defusing regional conflicts.<sup>19(p1)</sup> These goals are harmonious with Maslow's concepts and the needs for self-esteem, self-actualization, and security. Therefore, our national security strategy sets the implied task to focus our stabilization and reconstruction operations on developing methods to meet Maslow's hierarchy of needs within the state's population. Because the hierarchy of needs must be met in the order given, appropriate attention must be applied to meet each level before an individual or population can truly accept the higher needs. Stabilization and

reconstruction operational plans must incorporate both the ways and means of addressing all of Maslow's needs to return the population to a peaceful and productive society. To meet this implied task, a methodology must be developed to assess the population's ability to meet their needs based on Maslow's hierarchical concept.

## **ASSESSING STABILIZATION AND RECONSTRUCTION NEEDS**

There are numerous models, surveys, and evaluation methods currently used by military, governmental, international, and nongovernmental organizations to determine the needs of a state during stabilization and reconstruction operations.<sup>24</sup> While similarities exist among these survey and evaluation methods, they also are diverse in their content and scope. Each organization's survey and evaluation scheme is designed to evaluate the needs that their particular organization can provide: medical, nutritional, engineering, educational, political, financial, agricultural, etc. This is, of course, a reasonable approach for the individual organizations to identify and provide the support that is within their capacity. What is lacking is an overall assessment looking at the state as a whole based on Maslow's hierarchy of needs.

Interviews with individuals and communities, not just government officials, will lead to a more comprehensive and reasonable assessment of Maslow's needs. The "man on the street" type interviews are extremely effective in determining what the "common man" desires. From interviews in Afghanistan, Djibouti, and Honduras, average people have the same desires as most Americans. They want to have a job, earn a living, provide for their families, and give their children more than they had, with a brighter future. The coalition forces' provincial reconstruction teams in Afghanistan are successfully using community meetings to connect with the population. They are finding that some communities desire little more than shovels, axes, and wheelbarrows while others desire wells, textbooks, or crop seeds.<sup>25</sup>

It is easy to visualize that in many stabilization and reconstruction operations, the biological and physiological needs will be the primary requirement to be met. The states where we are currently involved in substantial stabilization and reconstruction operations,

Afghanistan and Iraq, previously were agricultural based economies. Iraq, prior to Saddam Hussein, was the world's sixth largest agricultural exporter; today it is a food dependent state. Coupled with its current dependence on the Oil for Food program, Iraqi agriculture lacks economic viability. Under Saddam, Iraq's agriculture productivity rapidly decreased due to lack of investment capital and poor land management, resulting in a requirement to import over 60% of its food.<sup>26</sup> Afghanistan, with limited natural resources, remains a subsistence agriculture state. In both of these states, as in the majority of other states where instability poses a threat to US interests, the state's economy and overall public health are closely tied to agriculture.

## **AGRICULTURAL ECONOMICS 101**

Food shortages leading to humanitarian disasters requiring international relief response are often thought to be the result of drought or other natural phenomenon. In part, they are, but the reality is that many states fail to provide adequate investment and resources to development of an agricultural economy, focusing instead on development of an industrial economy.<sup>27</sup> Interventions in the supply of food, while meeting the immediate needs of the at-risk population, lead to destabilization of the state's agricultural economy. Provision of free food supplies reduces the marketability of locally grown commodities, while governmental programs to keep consumer costs low bankrupts the producers.<sup>27</sup>

Promoting a strong world economy enhances US national security and advances prosperity and freedom throughout the world. Enhancing economic growth in a world where the majority of the population lives on less than \$2 a day allows people to meet their basic hierarchy of needs and lead healthier lives, creates jobs, and raises incomes. Lifting the veil of poverty stimulates social, economic, and legal reform, deters spoilers, and reinforces liberty.<sup>19(p17)</sup> The United States and other donor states have provided billions of dollars in developmental assistance to poor states and yet have failed to spur economic growth and, in many cases, have created dependent societies.<sup>19(p21)</sup> Often, the aid provided hindered the recipient state's economic development. The provision of free grain and other subsistence products destroys the market for locally produced products. In Afghanistan, the provision of free grain to the general population has destroyed the

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local grain market, forcing farmers to return to raising opium poppies as a cash crop. This is a case where a helping hand is a slap in the face. Cultivation of opium poppies continues the cycle of smuggling, trafficking, and organized crime in Afghanistan, reducing the ability of the central government to achieve an economic and political base for stabilization and reconstruction. A rapid influx of international and nongovernmental organizations into a destabilized state does not necessarily improve the ability of individuals to meet their basic needs. Frequently, these aid organizations compete with the local population for food and shelter and create inflation in the labor and housing markets, much to the detriment of the state's economic stabilization and reconstruction programs.<sup>10(pp92-96)</sup>

Historically, traditional societies were based on agriculture, today's modern societies are based on industry.<sup>28</sup> The transition from subsistence agriculture to the development and manufacture of microchips is an evolutionary process requiring many years of small sequential changes. Promoting agricultural projects promotes a sustainable development in developing or transitional states. For the majority of the western states, agriculture was the building block of their beginning. A state must be able to provide sustenance for its own populace before it can move on to becoming an international player. Today, third world states readily see what the West has to offer and want to leap forward into "instant Westernization." In their eagerness, they fail to realize that the West did not just happen in a year, or a decade; it took centuries for Western countries to achieve the wealth and prosperity they now enjoy. Westerners may have forgotten that our ancestors struggled through the crawl→walk→run stages of development to achieve our prosperous societies. Many Western organizations wish to bring the third world into the modern age without working through the growing pains, struggles, and self-satisfaction of the crawl→walk→run scenario. The United States, while extremely successful, was not born rich and famous—Americans worked for it, starting out as agriculturalists. Once they were capable of meeting their biological needs they progressed by moving to industrial enterprises and beyond.

Focusing on agricultural projects provides relief from dependency on donor countries to the farmer and his family, and creates long-term employment in

agricultural related occupations such as milling, processing, food production, and distribution. As a single industry, agriculture is the most capable of providing the population with a means of achieving its basic needs with minimal investment required from donor countries.

## **INTERRELATIONSHIP BETWEEN HUMAN AND ANIMAL HEALTH**

The complex interrelationships between human and animal health, transmission of disease, food production and processing, and economic health at all levels significantly affect the overall physical and economic health of a state. The raising and maintenance of livestock for food and milk production (camels, cattle, sheep, goats, swine, and poultry) and as transportation and labor (camels, cattle, and equines) is a vital component of the individual's and state's economy and public health. Even the poorest subsistence farmers regard livestock as key investments that will provide support through droughts and crop failure.

In developing and transition countries, animal husbandry is the largest single sector of agricultural economics, and, as the state develops, the importance of livestock increases.<sup>29</sup> In developing countries, much of the agricultural enterprise consists of family farms growing crops and raising livestock to provide their own subsistence. As a state develops, farmers and livestock producers must increase their production capabilities to meet the needs of a growing population.<sup>30</sup> There are many examples of agricultural-based programs in counter-insurgency operations. In the 1970s, the British Army was performing counter-insurgency operations in Oman. One of their successful programs was veterinary support to local cattle owners, improving their herds, providing wells to water them, and providing veterinary medical support. The condition of the area's livestock improved, resulting in increases in both the availability of food and amount of income. This in turn led to greater ability to purchase consumer goods, reducing insurgent recruiting among local population.<sup>21(pp217-218)</sup>

## **US ARMY VETERINARY CORPS**

The US Army Veterinary Corps does not include the performance of stability and reconstruction operations in its current mission statement.<sup>31</sup> However, Veterinary Corps personnel frequently are engaged in these

operations as members of special operations forces, civil affairs units, and civil-military operations task forces.

### **Utilization of Veterinary Corps Personnel**

Veterinary Corps personnel, in limited numbers, are assigned or attached to special operations forces and civil affairs units at various command levels. In these assignments, they work closely with host state's military counterparts and government ministries and agencies. Special operations forces veterinary assets assist in the planning and execution of population and resource control, civic action, humanitarian assistance and other security, development, and stability programs. In these positions, Veterinary Corps personnel perform assessments and collect data on the host state's available health and agricultural administration and operations infrastructure, developing support and assistance programs to expand the legitimacy of the host state's government.

Civil affairs Veterinary Corps assets frequently are involved in humanitarian and disaster relief programs in coordination with US governmental agencies, other DoD elements, coalition partner governmental agencies, international and nongovernmental organizations, and the host state ministries and agencies. Civil affairs veterinarians perform a wide range of public health and veterinary preventive medicine activities and programs in concert with the host state's ministries and agencies.

Veterinary Corps officers are versatile and capable of working closely with a wide variety of host state ministries, and governmental, international, and nongovernmental agencies. The Coalition Joint Civil-Military Operations Task Force-Kabul (CJCMOTF-Kabul) Veterinary Corps officers worked not only with the Afghan Ministry of Agriculture and Animal Husbandry, but also with the Ministries of Higher Education, Public Health, and Defense. They coordinated with international and nongovernmental organizations such as the United Nations' Food and Agriculture Organization and World Health Organization, the Dutch Committee for Afghanistan, and the Mayhew Animal Home of London on a variety of projects to improve the health of both the animal and human populations. Improvement of agricultural programs and national food production capabilities directly supports Maslow's hierarchy of needs.

During stability and reconstruction operations, veterinary service support operations to US and coalition forces include subsistence inspections to ensure safety, security, and wholesomeness; and the provision of veterinary medical services to military working dogs and other government-owned animals. The local procurement of food and water, providing adequate sources are available without inhibiting the availability to the local populace, is a means of stimulating the local economy. Veterinary service inspections of local commercial subsistence operations not only serves to determine if they are capable of meeting US procurement standards, but also provide the operators a set of goals to improve their facilities. While it is not currently in the scope of the Veterinary Corps to inspect commercial operations with the intent of providing guidance and training, that area should be explored. Veterinary Corps personnel could provide training in food industry good manufacturing processes, food sanitation and hygiene, inspection procedures and techniques, hazardous and critical control points program, and food handling and storage procedures. Improving the state's ability to produce safe and wholesome subsistence leads to an overall improvement in public health, reducing the burden on donors for food and health care.

Working alongside the host state government's ministries of agriculture, animal husbandry, and public health personnel, Army Veterinary Corps personnel design and execute local, regional, and national support programs to improve the health of the domestic animal population. Improving the health of the domestic animal population, while manpower intensive, is generally a cost effective method to improve the health of the human population. Reducing enzootic (animal diseases that circulate among and affect only the animal population) and zoonotic (animal diseases that circulate among the animal population and create disease in humans) diseases leads to an improvement in overall public health. Veterinary corps personnel, working with host state veterinarians, can develop animal vaccination programs, herd health programs, and animal husbandry programs. Healthier animals produce more meat and milk, have increased reproductive capacity, and cost less to maintain. These gains can be seen without a change in the availability of livestock feed and may even reduce the feed requirements based on productivity.

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Veterinarians frequently conduct domestic animal vaccination programs to reduce the prevalence of enzootic and zoonotic disease in support of host state governments. These programs provide a deep-rooted positive impression of the United States and its commitment to the host state's government. Currently, veterinary programs are ongoing in Afghanistan, Iraq, and the Horn of Africa region to improve animal health and provide training to local producers, veterinarians, and veterinary technicians. When available, these programs may be conducted in concurrence with international or nongovernmental organizations to increase the support provided.

In Honduras, mountain dwellers often traveled 2 days to bring their animals to Army Veterinarians who were providing vaccinations and deworming medications. When combined medical, dental, and veterinary services were offered in Honduran villages, it was the veterinarian who had the most patients and longest lines. When asked why they were more concerned with having their livestock immunized than their children, the Hondurans' standard reply was "it is easy to get more children, but I only have one horse (cow, goat, etc) and my family cannot survive without it."

Programs in Afghanistan initiated by the CJCMOTF-Kabul Veterinary Corps officers included reinvigoration of the Afghan Ministry of Agriculture and Animal Husbandry's veterinary infrastructure, rebuilding the national veterinary diagnostic laboratory and national vaccine laboratory; working with nongovernmental organizations to build veterinary clinics to improve access to veterinary services and to serve as veterinary training facilities. Other CJCMOTF-Kabul veterinary projects included rebuilding greenhouses to provide the population with garden plant starts for self-sufficiency; developing resources to improve dairy herd genetics; rebuilding the national animal and crop production research facilities; rebuilding the national poultry industry infrastructure; and providing supplies to regional veterinary clinics to service local populations.

Programs developed with the Afghan Ministry of Higher Education included rebuilding and providing water and electricity to the Schools of Veterinary Science and Pharmacy; provision of supplies and equipment to the Schools of Veterinary Science, Pharmacy, and Education; supplying animals and

teaching anatomy at the School of Veterinary Science; and developing a self-sustaining poultry cooperative at the veterinary school to both teach and feed students and faculty members. The CJCMOTF-Kabul veterinary officers also were responsible for assessments of Afghan medical facilities and coalition partners' medical support to the Afghan population, and collection and reporting of human and animal disease prevalence data. They also promoted infrastructure projects to improve crop irrigation, develop wells to water livestock, and roads to improve agricultural commerce.

### **MEETING MASLOW'S HIERARCHY OF NEEDS**

Army Veterinary Corps officers can design and conduct stabilization and reconstruction operations to support all of Maslow's hierarchy of needs. Increasing animal and crop food production and food safety increases the populations' ability to meet their biological and physiological needs. The presence of Army Veterinary Corps personnel working with host-state personnel in local communities is a sign of US military presence, stimulates faith and allegiance to the US-supported government, and supports safety needs. Maintaining or returning the population to rural environs where they can become self-sufficient, rather than in displaced persons camps or in urban slums without jobs or resources, improves the sense of belongingness and supports the concept of families working closely together for a common future. Supporting the ability of the population to provide for themselves and their families and reducing their daily dependence on donor organizations for their subsistence elevates their self-esteem. By meeting and sustaining the 4 lower hierarchical needs, individuals can now seek self-actualization, be that maintaining an agrarian lifestyle or developing other industries.

### **OTHER ORGANIZATIONS**

Many organizations, independently or as a coalition, develop and execute programs similar to those commonly developed by Army Veterinary Corps officers. However, those organizations generally are absent during armed conflict and do not return until the state's internal security has stabilized. As a part of the US force, the Veterinary Corps can operate in hostile environments where the early establishment of stabilization and reconstruction programs is critical to the overall outcome of the operation. Unlike some

international and nongovernmental organizations, Army Veterinary Corps personnel are not trying to establish dependency on outside organizations, but are supporting the host state and enhancing its legitimacy to the population.

## CONCLUSION

Stabilization and reconstruction operations are integral components of both peace and war, and therefore cannot be overlooked in the planning cycle for peacetime engagement and combat operations. In the war on terrorism, the United States must promptly address the internal concerns of troubled and failing states to reduce the potential for terrorist organizations to achieve a foothold in these states.<sup>32,33</sup> United States intervention in these failed or failing states must be proactive and address the population's basic needs. Focusing on agricultural production can be an efficient and cost-effective mechanism for early intervention.<sup>32</sup> The incorporation of agricultural programs, led by Veterinary Corps officers, into theater engagement plans may reduce the occurrence of a greater humanitarian crisis that could require a larger US military presence to alleviate.<sup>34</sup> The Army Veterinary Corps can provide substantial assistance as a leading component in stability and reconstruction operations to stimulate the agricultural systems and economy of the state. The inclusion of Veterinary Corps personnel in the early stages of operational planning and the early deployment of veterinary assets can improve the Joint Force Commanders' ability to negate spoilers within the population, and provide the population a means of self-sufficiency requiring less international and nongovernmental aid, and reduce the time required to complete such operations. The promotion and initiation of sustainable agricultural programs within the state will significantly improve the status quo and lead to overall national economic and social growth. Effective utilization of US Army Veterinary Corps officers in stabilization and reconstruction operations can reduce the possibility of a humanitarian crisis or mounting insurgency, and thus achieve the favorable security conditions required to protect the homeland and United States' interests around the world.

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